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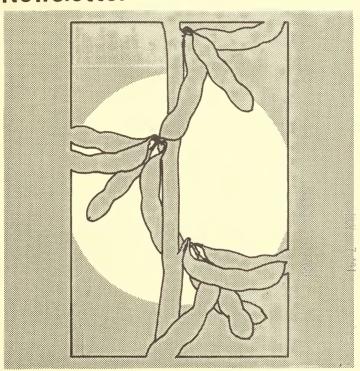
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Patricia

Soybean Genetics Newsletter



Volume 14

APRIL 1987

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Agricultural Research Service - USDA

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I. FOREWORD

Volume 14 of the SOYBEAN GENETICS NEWSLETTER is serving its original purpose of providing a forum for soybean scientists to exchange research information and data. Our own research group finds that we refer to the mailing list frequently for addresses of other scientists, and the Reference section proves invaluable upon occasion.

We would like to call your attention to the Second Edition of SOYBEANS: IMPROVEMENT, PRODUCTION, AND USES. This supplements, and in some ways, supplants the First Edition, published in 1973. This volume has been many years in the writing and production, and is due off the presses this spring. J. W. Wilcox, USDA ARS, of Purdue University, is the editor; the authors of the separate chapters are all leaders in their particular aspects of soybean science. The Announcements section of this newsletter lists where to inquire for purchase.

Also in the Announcements section, you will find a form with which to express your intention to attend the WORLD SOYBEAN RESEARCH CONFERENCE IV, to be held in Buenos Aires, Argentina, in March of 1989. It may seem early, but an international conference of such a broad scope requires years of planning and organizing.

Graduate students and technicians who made this newsletter possible are Sandra Benavente, Susan Blomgren, Brad Hedges, Anita Bries, Holly Heer, Rhonda Honeycutt, Gretchen Willis, and Zhang Fan. Without their volunteer assistance, it never would have gotten off the ground.

We usually neglect to show our gratitude to the best typist in the world, Mrs. Donna Gladon, whose ability and expertise give our pages the precision and elegance we are proud of. Her patience is amazing.

We continue to have papers on research in isozymes. The exciting new fields of tissue culture, molecular biology and mobile genetic elements are represented by research articles in this volume, as are 13 different countries. This is our biggest issue so far, and, we hope, our best.

The data presented in the Soybean Genetics Newsletter are not to be used in publications without the consent of the respective authors.

Mention of a trademark or proprietary product by the USDA or Iowa State University does not imply its approval to the exclusion of other products that may also be suitable.

Announcement

THE SECOND EDITION OF THE SOYBEAN MONOGRAPH

Soybeans: Improvement,
Production, and Uses

WILL COME OFF THE PRESSES THIS SPRING.
INFORMATION ON PURCHASE OF THIS BOOK
MAY BE OBTAINED FROM:

Sherri Mickelson, Managing Editor American Society of Agronomy 677 South Segoe Road Madison, WI 53711 (608) 273-8080

Anyone planning to attend the World Soybean Research Conference IV could express their intention with this form, mailing it to:

Association Argentina de la Soja Av. Corrienties 127 - 3er Piso 1043 - Buenos Aires Republica Argentina

IV CONFERENCIA MUNDIAL DE INVESTIGACION EN SOJA WORLD SOYBEAN RESEARCH CONFERENCE IV BUENOS AIRES - REPUBLICA ARGENTINA

Marzo/March - 1989

INTENCION DE ASISTENCIA/INTENTION TO ATTEND

Anyone wishing information concerning the NINTH AUSTRALIAN PLANT [] [] [] BREEDING CONFERENCE, to be held in Wagga Wagga, New South Wales, [] 27 June - 1 July, 1988, should contact Dr. Barbara Read, [] [] [] Secretary, APBC, Agricultural Research Institute, Private Mail [] [] Bag, Wagga Wagga, NSW, Australia, 2650.

III. ERRATA

1. Soybean Genetics Newsletter 12:82-86. 1985.

T280 was listed as the Genetic Type Collection number for nonfluorescent root mutant fr5 fr5. This was an error. The correct T number is T285. See Crop Science 27:62-65, 1987, for the complete description of T285 (fr5). T280 is the correct Genetic Type Collection number for C1640, a low linelenic acid mutant with the gene symbol fan.

2. Soybean Genetics Newsletter 13:176-182.

Measured and predicted yield response of soybeans to simulated acid rain. F. L. Allen and H. L. Bhardwaj. The chart accompanying this article was erroneously excluded, and was printed instead on page 168. The article with chart are reprinted in entirety in this volume on pages 148-155.

IV. SOYBEAN GENETICS COMMITTEE REPORT

Minutes of the Meeting

The Soybean Genetics Committee met Monday, Feb. 23, 1987, at the Ramada Inn, Memphis, TN. This meeting was held in conjunction with the annual Soybean Breeders Workshop.

Committee members in attendance were R. L. Bernard, W. D. Beversdorf, Y. T. Kiang, J. H. Orf and J. R. Wilcox. Also present were B. A. McBlain, J. Specht and B. D. Rennie. G. R. Bowers and B. A. McBlain have been elected to three-year terms on the Committee, replacing W. D. Beversdorf and J. R. Wilcox. Current Committee members and the expiration date of their terms are as follows:

- R. L. Bernard (Ex officio)
 (Curator of soybean genetics collection)
 Department of Agronomy
 University of Illinois
 1102 South Goodwin St.
 Urbana, IL 61801
 (217) 333-4639
- G. R. Bowers (1990)
 Texas Agric. Exp. Station
 Route 7, Box 999
 Beaumont, TX 77706
 (409) 752-2741
- R. I. Buzzell, Chairman (1988) Agriculture Canada Res. Station Harrow, Ontario Canada NIG 2Wl (519) 738-2251
- X. Delannay (1988) Monsanto Agricultural Co. 700 Chesterfield Village Pkwy. St. Louis, MO 63198 (314) 694-1000

- Y. T. Kiang (1989) Dept. of Plant Sciences and Genetics University of New Hampshire Durham, NH 03824 (603) 862-3218
- B. A. McBlain (1990) Department of Agronomy, OARDC Ohio State University 1680 Madison Ave. Wooster, OH 44691-6900 (216) 263-3878
- J. H. Orf (1989) Dept. of Agronomy and Plant Genetics University of Minnesota St. Paul, MN 55101 (612) 625-8275
- R. G. Palmer (Ex officio)
 (Curator cytogenetic collection and
 Editor of Soybean Genetics
 Newsletter)
 Departments of Agronomy and Genetics
 Iowa State University
 Ames, IA 50011
 (515) 294-7378
- Dr. R. I. Buzzell was elected chairman of the Committee for 1987. Manuscripts concerning qualitative genetics interpretation and gene symbols should be sent to him for review.

In order to reduce the time required to review and return manuscripts, the following changes will be made in the procedures used by the Committee:

Reviewers of manuscripts will be given a deadline of two weeks to return the reviewed manuscript to the Chairman (who will then get it to the author as soon as possible). If the reviewer has not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation.

- 2. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol." The manuscript will not be reviewed for style except as it influences the clarity of interpretation. Authors may submit unpublished (but comprehensible) manuscripts for review. This should reduce some of the delay that accompanies production of an article.
- 3. Gene symbols will only be approved in cases where the relevant material is made available to the soybean germplasm collection. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretations does not depend on symbols, in most cases). The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is unnecessary.

Minutes

In discussion, it was agreed that the Committee had been inefficient in dealing with manuscripts in the past. It was moved (Orf/Bernard), and passed, that deadlines should be set to speed up reviews (see above). In addition, a form will be drafted for assisting the reviewers in assessing the manuscripts.

It was agreed (Wilcox/Orf) that no symbols will be assigned if material has not been submitted to, or is not currently available in, the soybean germplasm collection. In the case of materials identified as possessing two or more "new" loci or alleles, lines isolating single loci are preferred.

The committee appointed to review the assigning of symbols for transformed material reported several proposals for symbols. However, the Committee felt that since these sorts of material will not be available in the immediate future, no decision was necessary at this time. The Committee will be monitoring the progress made in tomatoes and other crops that are being transformed to assist it in making a decision.

It was agreed (Orf/Kiang) that the Committee should publish a complete list of approved soybean gene symbols in the Soybean Genetics Newsletter in 1987 and every 5 years thereafter. A cumulative update of new symbols would be published between the full updates.

A discussion occurred on the potential to involve more researchers from other countries in the use of a single set of gene symbols. Submission of manuscripts from distant lands would not be practical, but an attempt will be made to contact a few researchers from each geographic region in order to foster cooperation in this field.

The Committee reaffirmed its stand to retain the original symbol approved for a gene and its alleles. Discussion of dominance and codominance was ruled less important than reducing confusion. It was suggested that authors only use those symbols appearing in the official list. However, if other symbols have appeared in the literature, these could be referred to for clarity.

Guidelines on the Evidence Necessary for the

Assignment of Gene Symbols

Researchers are strongly encouraged to send all gene symbols and genetic interpretations to the Soybean Genetics Committee for review prior to publication to avoid duplication and/or confusion.

The following is a set of guidelines prepared by the Soybean Genetics Committee and intended to help researchers undertaking genetic analysis of soybean traits. Of necessity, these procedures will often need to be modified by the researcher to fit the specific situation, but an application of these guidelines should aid in making the correct genetic interpretation.

- 1. A genetic hypothesis is made on the basis of classification of segregating progeny, usually the ${\rm F}_2$ generation and here called the hypothesis generation.
- 2. A second generation is classified to confirm the proposed genetic hypothesis. This second generation may be progeny of the hypothesis generation (usually ${\rm F_3}$) or progeny of a testcross (${\rm F_1}$ x recessive homozygote).
- 3. Traits that are strongly influenced by nongenetic factors require verification of the classification scheme by evaluation of the progeny from homozygous plants of the hypothesis generation. Testcross data are not suitable for this purpose.
- 4. For genes controlling a phenotypic expression similar to that of previously published genes, data must be obtained to test for uniqueness and allelism. This will usually require crossing a homozygous line carrying the newly identified gene with the original sources of the previously published genes.
- 5. Follow the guidelines (Rules for Genetic Symbols) published in the Soybean Genetics Newsletter to assign the symbol.
- Submit the manuscript to the chair, Soybean Genetics Committee, for review of the genetic interpretation and approval of the gene symbol (see Soybean Genetics Newsletter for name and address).
- 7. If the line in which the new gene occurs is not already in the USDA Germplasm Collection, send a seed sample of the line to the curator of the Genetic Type Collection for assignment of a T-number and maintenance of the seed (see current Soybean Genetics Newsletter for name and address).

References

- Mather, K. 1951. The measurement of linkage in heredity. Methuen & Co., Ltd., London. John Wiley & Sons, Inc., New York.
- Hanson, W. D. 1959. Minimum family size for the planning of genetic experiments. Agron. J. 51:711-715.
- Sedcole, J. R. 1977. Number of plants necessary to recover a trait. Crop Sci. 17:667-668.

Rules for Genetic Symbols

1) Gene Symbols

- a) Gene symbols should not be assigned to traits for which no inheritance data are presented.
- b) A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.
- c) Genes that are allelic shall be symbolized with the same base letter(s) so that each gene locus will be designated by a characteristic symbol base.
- d) Gene pairs with the same or similar effects (including duplicate, complementary or polymeric genes) should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1 . Y_2 , etc.) The numerals may be written on the same line as the base. (Example: Y1, Y2, etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.
- e) The first pair of alleles reported for a gene locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: Ab, ab. Ab is allelic and dominant to ab.)
- f) If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles or those symbolized subsequently to the pair first published shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: R, r^m, r.) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (For example, Rpsl-b, Rpsl-k, and Ap-a, Ap-b, Ap-c.) The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

If independent mutations with the same or similar phenotype are identified at the same locus, until it is possible genetically to ascertain if they represent identical or separate alleles, the gene symbol should be followed by an identifying designation in parentheses. The identifying designation, which should NOT be in italics or underlined, can be the place where the mutation was found, the cultivar in which it was found, or any other relevant characteristic of the mutation. (Example: ms1 (Tonica), or ms1 (Ames 2).) This will ensure that possible subtle differences between the mutations, such as differences in DNA sequence, or unique pleiotropic side effects, are not overlooked by workers using those genes.

- g) Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.
- h) The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clearcut. The decision for intermediate cases is at the discretion of the author, but should be in accordance with previous practices for the particular type of trait.

The following sections concern supplementary symbols that may be used whenever desired as aids to presentation of genetic formulas.

- i) An underscore may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)
- j) A question mark may be used in place of a symbol when the locus or allele is unknown or doubtful. The name of the line in which the gene was identified should be included in the symbol, in parentheses. A hyphen preceding the question mark indicates an unknown allele at a known locus, the absence of a hyphen indicates an unknown locus. (Example: Rps? (Harosoy) an allele in Harosoy at an unknown locus or Ap-? (T160) an unknown allele in T160 at the Ap locus.
- k) Plus symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II) Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

- a) A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. The example, Adh (alcohol dehydrogenase); Idh (isocitrate dehydrogenase). The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.
- b) The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors.
- c) Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to ensure that subsequent work corresponds to the original study.

III) Linkage and Chromosome Symbols

- a) Linkage groups and the corresponding chromosomes shall be designated with arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.
- b) Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv inversions, and Tri primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second as Def 1b, etc. The first published inversion in chromosome 1 shall be designated with the arabic numeral that corresponds to its respective linkage group number.
- c) Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran l would represent the first case of a published reciprocal translocation; Tran 2, the second case, etc. The first published deficiency shall be symbolized as Def A, the second as Def B, etc. The first published inversion shall be symbolized as Inv A, and the second as Inv B, etc. The first published trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

IV) Cytoplasmic Factor Symbols

a) Cytoplasmic factors shall be designated with one or more letters prefixed by cyt-. (Example: cyt-G indicates the cytoplasmic factor for maternal green cotyledons, cyt-Y indicates that for maternal yellow cotyledons.)

V) Priority and Validity of Symbols

- a) A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.
- b) In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

VI) Rule Changes

 These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

V. USDA NORTHERN SOYBEAN GERMPLASM COLLECTION REPORT January 1987

In 1986, a total of 314 seed requests for 13,021 seedlots were filled from the USDA Northern Soybean Germplasm Collection. U.S. researchers in 31 states requested 12,020 of these seedlots, with the remaining 1001 requested by foreign researchers in 26 countries.

Of the 8000 strains in the Urbana collection, 660 were grown in 4-row plots in 1986 for germplasm maintenance at Urbana. In addition, 23 accessions were added to the collection after the 1986 harvest. Of these, 19 were from China, 2 from France, and 2 from Sweden. These are now available for distribution.

The number of accessions maintained at Urbana is listed below by maturity group and by country of origin.

^{*}Africa, Australia, and Latin America.

There were no new additions to the wild soybean collection in 1986, and its current status is given on the following page.

Country of origin	000	00	0	н	II	III	IV	Λ	VI	VII	VIII	IX	×	Total	Percent
China	11	11	21	12	31	13	15	8	9	1	0	0	0	132	19.6
China, Taiwan	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0.3
Japan	0	0	0	0	0	1	7	51	85	46	0	3	0	193	28.6
S. Korea	0	0	0	0	2	0	40	244	27	1	0	0	0	314	46.5
USSR	0	17	7	5	5	0	0	0	0	0	0	0	0	34	5.0
Total	11	28	28	17	38	14	61	303	121	48	0	3	2	675	100.0

In January 1986, 1417 soybean accessions were received from the National Institute of Agrobiological Resource in Tsukuba, Japan. During the summer, 1238 soybeans were grown at Urbana (the remaining 179 were from southern Japan and were grown at Stoneville) and compared to 504 PI strains from Japan with similar names. Duplicate accessions (253) were identified and discarded. PI number assignment has been requested for the remainder. Seeds will be available after the 1987 harvest. In addition, 2 new accessions were grown in 1986, one from Sichuan, China, and one from Seoul, South Korea.

New soybean accessions received in 1986, which will be planted for the first time in 1987, include 31 from AVRDC, Taiwan, China, 2 from Sri Lanka, and 59 from the Vavilov All-Union Institute of Plant Industry, Leningrad, USSR. New wild soybean accessions received for 1987 planting include 89 from the National Institute of Agrobiological Resources, Tsukuba, Japan; 129 from the Vavilov All-Union Institute of Plant Industry, Leningrad, USSR; 1 from South Korea; and 4 from Iwate, Japan.

We also received lll accessions of the 12 perennial species of *Glycine* from Tony Brown, CSIRO, Australia. This forms a "core collection" of this subgenus which Dr. Brown has selected from his many accessions to be representative of both the genetic and geographic diversity. When facilities (greenhouse space) become available, we will increase these and make them available to researchers.

The first year of a 2-year germplasm evaluation was conducted in 1986. A total of 510 PI accessions from 446.893 to 486.355 and 34 varieties released since 1983 were evaluated for 31 descriptors. In cooperation with Dr. James Orf, Maturity Groups 000, 00, and 0 (61 lines) were grown at the University of Minnesota. Maturity Groups I to IV (483 lines) were evaluated at Urbana, Illinois. A second and final year of the evaluation will be conducted in 1987.

An evaluation of Maturity Groups 000 through IV of the USDA Soybean Germplasm Collection (2795 accessions from PI 270.000 to 427.107 and 93 named varieties released from 1966 to 1980) by Dr. Randall Nelson will be published as USDA Technical Bulletin 1718 in early 1987. These data are also in the

USDA Germplasm Resources Information Network (GRIN). The 1965-69 evaluation reports (2016 FC and PI strains to PI 270.000 and 174 old domestic cultivars) are still available from the curator. Data include origin, descriptive data, yield, lodging, height, seed size, and composition, etc. and are in GRIN. GRIN now has descriptive and evaluative data on about 300 northern domestic varieties and over 6000 northern FC and PI strains (through PI 445.845).

Annually updated checklists and other printed information are available from the curator. These include:

- U.S. and Canadian Germplasm Variety Checklist (140 strains with maturity group and descriptive codes) January 1987;
- 2. U.S. and Canadian Public Variety Checklist (169 strains with maturity group and descriptive codes) January 1987. Tables with pedigrees and release information are in preparation;
- FC and PI Strain Checklist (7027 strains with maturity group) March 1987; An inventory with origin information is nearing publication;
- 4. Wild Soybean Checklist (675 strains with maturity group) January 1986;
- Wild Soybean Inventory (675 strains with maturity group and passport data) January 1986;
- Genetic Type List (107 strains, up to T287, with genotype, phenotype, and origin information) March 1987;
- Genetic Isoline (300 strains wtih genotype, phenotype, and origin information) March 1975; (soon to be revised);

An international directory of soybean germplasm collections was published early in 1986 in conjunction with the International Board of Plant Genetic Resources (IBPCR) and the International Soybean Program (INTSOY). This directory includes information on 87 soybean germplasm collections (cultivated, wild, and perennial species) in 43 countries. Copies of this publication will be available from INTSOY, University of Illinois, 113 Mumford Hall, 1301 West Gregory Drive, Urbana, Illinois, 61801 U.S.A.

R. L. Bernard and G. A. Juvik USDA-ARS University of Illinois W-321 Turner Hall 1102 S. Goodwin Urbana, IL 61801

VI. USDA SOUTHERN SOYBEAN GERMPLASM REPORT

Report on germplasm in Maturity Groups V to X - February 1987

Maturity group	Total entries 1983	Total entries 1984	Total entries 1985	Total entries 1986
V	1520	1549	1550	1550
VI	470	482	486	487
VII	334	346	349	351
VIII	285	297	303	308
IX	124	131	143	149
X	151	154	_158	165
	2884	2959	2989	3010

²⁹ New additions for 1986.

Stoneville will grow 351 lines of germplasm VII in 1987 for increase.

Stoneville will grow 643 lines of 4 plants each for single-row selection.

1986 seed requests filled: 7,265 packets, to 21 countries, and to 32 states.

Edgar E. Hartwig Calton Edwards USDA ARS Stoneville, Mississippi

VII. EXECUTIVE BOARD OF COMMERCIAL SOYBEAN BREEDERS - 1987

Nancy Seburn, chairman Dekalb-Pfizer Genetics P.O. Box 8, Blacktop T29 Beauman, IA 50609 (515) 366-2606

Tom Wofford, vice-chairman Delta and Pine Land Company P.O. Box 1118, 1200 Herring Ave. Stuttgart, AR 72160 (501) 673-8365

Roger McBroom, secretary Northrup King & Company 306 Meadow Dr., Box Z St. Joseph, IL 61873 (217) 469-2746 Alan Walker Asgrow Seed Company Rt. 1, Box 1916 Redwood Falls, MN 56283 (507) 644-3011

Dennis Byron Jacques Seed Company 720 St. Croix Prescott, WI 54021 (715) 262-3226

Ervin Mueller Pioneer Hi-Bred International Flippin Lane, Box 649 Union City, TN 38261 (901) 885-4882

VIII. THE GERMPLASM RESOURCES INFORMATION NETWORK: EASY ACCESS TO SOYBEAN GERMPLASM DATA

The Germplasm Resources Information Network (GRIN) is a computer database maintained by the USDA Agricultural Research Service (ARS). It is a central repository for detailed descriptive information for most plant germplasm maintained within the National Plant Germplasm System. This information includes: various types of passport (or introduction) data; complete and recently verified taxonomic nomenclature and geographic standards; the ARS-funded location at which the germplasm is stored and maintained as well as associated inventory data; characterization and evaluation data including environmental conditions under which trials were conducted and publication citations concerning these trials; and descriptors and code values used with the crop description information. Twenty-four germplasm maintenance sites participate by updating, adding, and deleting information through telecommunication access to the central computer located in Beltsville, Maryland. This keeps all data current and leaves responsibility for its integrity to those best suited to determine its validity.

Direct access to this information is possible for any plant scientist within the United States, Canada, and Mexico, as well as scientists affiliated with international agricultural research centers. A "user-friendly" interface is available to assist researchers with little or no computer experience in obtaining the information they desire. Users can also take advantage of data manipulation software to select only the germplasm that fits their specific criteria. During a computer session, users may submit orders for this germplasm directly to the responsible maintenance site. After obtaining an access code from the GRIN Database Management Unit, individuals only need a computer terminal or personal computer with telecommunications ability to access GRIN.

At this time, GRIN contains some data for all soybean accessions held at both the Northern Soybean Collection, Urbana, Illinois, and the Southern Soybean Collection, Stoneville, Mississippi. Taxonomic nomenclature exists for 24 species in the *Glycine* genus. Passport information is available for 7,389 and 2,980 samples held at the Northern and Southern collections, respectively. Duplicates for this germplasm are stored at the National Seed Storage Lab in Ft. Collins, Colorado. Characterization and evaluation data are available for 9,621 samples.

For assistance and/or additional information on GRIN, or to obtain an access code and user documentation, please contact:

Database Manager USDA/ARS/PGGI/GRIN, BARC-W Building 001, Room 130 Beltsville, MD 20705, USA Telephone: 301-344-1666

> M. C. Perry M. A. Bohning Univ. of Maryland and USDA-ARS

SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee held its annual meeting February 23, 1987 at the Soybean Breeders' Workshop in Memphis, Tennessee. Twelve of the 14 members were in attendance. Also attending was Mark A. Bohning, Crop Advisory Committee Facilitator, Plant Genetics and Germplasm Institute. Those elected to three-year terms were: Kuell Hinson, USDA-ARS, University of Florida, Gainesville; Clark Jennings, Pioneer Hi-Bred International, Waterloo, Iowa; and Donald Schmitt, North Carolina State University, Raleigh.

Updates on both the northern and southern portions of the USDA Soybean Germplasm Collection were given by Richard Bernard and Calton Edwards, respectively. Those reports are presented in the Soybean Genetics Newsletter, pages 11-14. A large collection (1417 accessions) of soybean germplasm was received from Japan in 1986. Urbana received a budget increase in FY 87 for acquisition, maintenance, and general evaluation of the collection.

The subject of availability of soybean germplasm data from the Germplasm Resources Information Network (GRIN) was discussed. Mark Bohning reported that GRIN is now available to the public. User manuals for accessing the soybean germplasm data base in GRIN are available by a request to: Jimmy D. Mowder, Database Manager, USDA, ARS, Room 130, Building 001, BARC-WEST, Belts-ville, MD 20705.

It was reported that a special two-hour symposium on GRIN will be given at the 1987 Annual Meetings of the American Society of Agronomy.

In a discussion of curator needs, Richard Bernard expressed concern about the maintenance of the perennial *Glycine* collection. There is a strong need for greenhouse space for this purpose. The use of tropical/subtropical areas to maintain the perennials was discussed. Puerto Rico, St. Croix, and other locations including Australia were considered. Concerns about growing the perennials at outlying locations were outcrossing, disease problems, technical knowledge of the personnel at the locations and the continuity of maintenance by cooperators.

Richard Bernard suggested that more effort needs to be made by authors to more completely identify foreign cultivars listed in papers in scientific journals. The name of the cultivar and the institution from which it was received should be given. Also, it would be desirable for cultivars being used in U.S. breeding and research to be offered for inclusion into the U.S. PI Collection, if not already included.

The Soybean Germplasm Crop Advisory Committee Bylaws were published in the Soybean Genetics Newsletter 13:31-32, 1986. These were reviewed and no changes were recommended. However, because of the close cooperation between many U.S. and Canadian scientists, a motion was passed to invite a soybean breeder from Canada to attend the 1988 committee meeting as an observer.

The duties and responsibilities of Crop Advisory Committees were published in the Soybean Genetics Newsletter 13:25-26. These were reviewed and no changes were made.

A discussion of the journal DIVERSITY emphasized the importance of encouraging CAC members to subscribe and to promote it to nonmembers. The journal is an excellent means to become better informed on germplasm issues.

Following are the current committee members, addresses, areas of representation, and dates of expiration of current terms.

Name	Address	Area of representation	Expiration of term
T. Scott Abney	USDA, ARS Dept. of Plant Pathology Purdue University W. Lafayette, IN 47907	Plant Pathology	1989
R. L. Bernard	USDA, ARS Dept. of Agronomy University of Illinois 1102 S. Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex offici
Edgar E. Hartwig	USDA, ARS Soybean Prod. Research P.O. Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex offici
Kuell Hinson	USDA, ARS Dept. of Agronomy University of Florida 1 FAA Bldg. 63 Gainesville, FL 32611	Public Breeding, South	1990
Clark Jennings	Pioneer Hi-Bred Int'l 3261 W. Airline Hwy. Waterloo, IA 50703	Private Breeding, North	1990
Thomas C. Kilen	USDA, ARS Soybean Prod. Research P.O. Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex offici
Philip Miller	USDA, ARS Beltsville Agric. Res. Center Bldg. 005, BARC-W Beltsville, MD 20705	USDA National Program Staff	ex offici
Randall Nelson	USDA, ARS Dept. of Agronomy University of Illinois 1102 S. Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex offici
J. H. Orf	Dept. of Agronomy and Plant Genetics University of Minnesota St. Paul, MN 55108	Public Breeding, North	1988

Current committee members (continued)

Name	Address	Area of Representation	Expiration of term
Reid G. Palmer	USDA, ARS G 301 Agronomy Iowa State University Ames, IA 50011	Cytogenetics and Molecular Genetion	1988 cs
Donald P. Schmitt	Dept. of Plant Pathology Box 7631 North Carolina State Univ. Raleigh, NC 27695-7631	Nematology	1990
J. Grover Shannon	Asgrow Seed Company P.O. Box 210 Marion, AR 72364	Private Breeding, South	1989
M. J. Sullivan	Edisto Experiment Station P.O. Box 247 Blackville, SC 29817	Entomology	1988
Richard Wilson	414 Williams Hall North Carolina State Univ. Raleigh, NC 27695-7620	Physiology	1989

Thomas Kilen was reelected as chairman of the committee and Clark Jennings was reelected vice-chairman. Both will serve one-year terms.

Thomas C. Kilen, Chairman Soybean Germplasm Crop Advisory Committee



SUMMARY OF LOCUS-TO-LOCUS LINKAGE ASSAYS IN SOYBEAN (Glycine max (L.) Merr.)

In 1984, a summary of locus-to-locus linkage data was published in the Soybean Genetics Newsletter (Yee and Palmer, 1984; reference 107). This list was not complete, although it did bring together information from a wide number of sources. A great deal of information on soybean linkage assays exists in the literature, or in manuscripts in preparation. The current article is designed to combine all of the available information of linkage assays performed on soybeans. Information on the 172 pairs assayed by Yee and Palmer (1984) has not been reproduced. The 495 pairs reported here bring the total number of soybean loci pairs assayed for linkage to 667.

Since this article was compiled by a small number of people, there are liable to be errors and omissions. However, the authors did their best to be thorough and accurate. If you are aware of any omissions, please submit them to the Soybean Genetics Committee for inclusion in a subsequent list. The listing of a linkage assay in this article does not imply agreement with the results. This article merely repeats data from the literature, or in manuscripts submitted to the Soybean Genetics Committee.

In order to save space, only a single assay by a single author is reported for unlinked loci. The assay reported was that which involved the largest population. Assays that involve unlinked loci, and were first reported in Yee and Palmer (1984), were not reported here, even when this work was done by a different group of researchers.

The symbols for the soybean loci start with either upper or lower case letters: symbols starting with upper case letters refer to isozyme or protein loci (e.g., adhl, Ep), while symbols starting with lower case letters refer to all other loci (e.g., ab, rpsl).

There are two designations used which are abbreviations for Phytophthora and nodulation response, These are Rps? and Rj?. Refer to the original reference for a more complete description of these designations.

B. D. Rennie University of Guelph

R. G. Palmer - USDA ARS
Iowa State University

Locus-to-locus linkage assays in soybean

Loci	Linkage ^l group	Ratio evaluated	Population ² size	Map 3 distance	Ref.
ab - del - g - i - w1 - y8 - y9 - y10	(,7) (,8)	9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1	no data shown		82 82 82 82 82 82 82 82
Aco1 - Ap - Dia1 - Enp - Ep - Idh1 - Idh2 - Sp1 - t - w1	(,9) (,12) (,1) (,1) (,8)	4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1	277+ 272+ 272+ 277+ 195* 276+ 113+ 276+ 212* 180*		35 35 35 35 35 35 35 35 35 35
Aco2 - Adh1 - Ap - Dia1 - Enp - fr1 - Idh1 - Idh2 - Sp1	(,8) (,9) (,12) (,1) (,8)	6:3:3:2:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1	220 220 126 219 432 432 399 317 389 124 128 314		28 28 35 28 35 35 35 35 35 35 35 35 35
Aco3 - Aco1 - Aco2 - Aco4 - Ap - Dia1 - Enp - Idh2 - Sp1	(1,) (1,) (1,) (1,9) (1,) (1,) (1,) (1,1)	4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:2:1:1:1:1:1 4:2:2:2:2:2:1:1:1:1:1 4:2:2:2:2:2:1:1:1:1:1 4:2:2:2:2:2:1:1:1:1:1 4:2:2:2:2:2:1:1:1:1 4:2:2:2:2:2:1:1:1:1 4:2:2:2:2:2:1:1:1:1 6:3:3:2:1:1	266 ⁺ 218, 222 ⁺ 117 ⁺ 207, 468, 249, 527, 192, 147	6% 31% 29%	35 35 35 35 35 35 35 35 35 35 35 35
Aco4 - Aco1 - Aco2 - Adh1 - Ap	(,8) (,9)	4:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1	277 ⁺ 397 221 220 320		35 35 28 28 35

Locus-to-locus linkage assays (continued)

Loci		Linkage ¹ group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
Aco4 -	· Dial		4:2:2:2:1:1:1:1	220		28
			4:2:2:2:1:1:1:1	234_		84
			4:2:2:2:1:1:1:1	315+		35
_	· dtl	(,5)	6:3:3:2:1:1	206		84
-	Enp		4:2:2:2:1:1:1:1	203 460 ⁺		85
			4:2:2:2:1:1:1:1			35
	Ep	(,12)	6:3:3:2:1:1	211*		35
	fr1	(,12)	6:3:3:2:1:1	410		35
	fr2		6:3:3:2:1:1	314		35
-	Idh1		4:2:2:2:1:1:1:1	331_+		88
			4:2:2:2:2:1:1:1:1	319		35
	Idh2		4:2:2:2:2:1:1:1:1	399 ^T		35
	ln	(,4)	6:3:3:2:1:1	212		84
	pb	(1)	6:3:3:2:1:1	303*		35
	Sp1	(,1)	6:3:3:2:1:1	319 ^T		35
_	t	(,1)	6:3:3:2:1:1	235		84
	7	(0)	6:3:3:2:1:1	306*		35
_	w1	(,8)	6:3:3:2:1:1	375		84
	7 7	(6)	6:3:3:2:1:1 6:3:3:2:1:1	205 * 299		35 46
	y11 — — — —	(,6) 				
Adhl -	Adh2	(8,8)	9:3:3:1	446	2%	32
-	Amy1	(8,)	'independent'	no data shown		31ъ
-	Dial	(8,)	6:3:3:2:1:1	183		32
	Gpd	(8,)	9:3:3:1	151		32
	Idh1	(8,)	9:3:3:1	95		32
	Idh2	(8,)	9:3:3:1	95		32
	Lap1	(8,9)	6:3:3:2:1:1	110		32
	Mpi	(8,)	6:3:3:2:1:1	105	30.5%	32
	Sp1	(8,9)	6:3:3:2:1:1	263		32
	Sp1	(8,)	9:3:3:1	263		31b
	Sod1	(8,)	9:3:3:1	123	0.1 %	31b
	w1 — — — —	(8,8) 	9:3:3:1 	1375	21%	50 — — —
Adh2 -	Amy1		'independent'	no data shown		31ъ
-	Sp1	(8,1)	9:3:3:1	183		31ъ
-	Sod1		9:3:3:1	123		31ъ
 Amy2 -	 Dial		4:2:2:2:1:1:1:1	415		51
Ap -	 Dial	(9,)	6:3:3:2:1:1	112		32
-	Gpd	(9,)	6:3:3:2:1:1	79		31a
-	Idh1	(9)	6:3:3:2:1:1	216		32
-	Idh1	(9,)	6:3:3:2:1:1	216		32
	Idh3	(9,9)	4:2:2:2:1:1:1:1	134	27.0%	32
	Mpi	(9,)	4:2:2:2:1:1:1:1	82		31a
	Pgi	(9,)	4:2:2:2:1:1:1:1	101		32
	Pgm1	(9,)	4:2:2:2:1:1:1:1	163		32
-	Pgm2	(9,)	4:2:2:2:2:1:1:1:1	145		31a

Locus-to-locus linkage assays (continued)

Loci	Linkage l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
Ap - Ti	(9,9)	4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1:1		9%	21
	(9,9) (9,9)	4:2:2:2:2:1:1:1:1:1		7% 16%	45 44
- w1	(9,8)	6:3:3:2:1:1	273	10%	67
 Cgy1 - Cgy2		4:2:2:2:2:1:1:1:1	82		23
- Cgy3		4:2:2:2:1:1:1:1			23
 Cgy2 - Cgy1		4:2:2:2:2:1:1:1:1	82		23
- Cgy3	(#)	4:2:2:2:2:1:1:1:1	82	'linked'	23
Cgy3 - Cgy1		4:2:2:2:1:1:1:1			23
- Cgy2	(#)	4:2:2:2:1:1:1:1	82 	?% 	23
Dial - Dia2		4:2:2:2:2:1:1:1:1			32
- Dia3		6:3:3:2:1:1	147		32
- Gpd		6:3:3:2:1:1	290		32
- Idh1		4:2:2:2:2:1:1:1:1			88
		4:2:2:2:1:1:1:1			51
- Idh2		4:2:2:2:2:1:1:1:1			88
		4:2:2:2:1:1:1:1			51
- Idh3		9:3:3:1	256		32
- Lap1	(,9)	4:2:2:2:1:1:1:1			51
- Mpi		4:2:2:2:2:1:1:1:1			32
- Pgd1		4:2:2:2:2:1:1:1:1			32
- Pgm1		4:2:2:2:2:1:1:1:1			88
		4:2:2:2:1:1:1:1			51
- t - w1	(,1) (,8)	6:3:3:2:1:1 6:3:3:2:1:1	406 173		51 49
			129		 32
Dia2 - Dia3 - Gpd		6:3:3:2:1:1	106		32
- Gpa - Idh1		6:3:3:2:1:1	119		32
- Idh2		6:3:3:2:1:1	105		32
- Idh3		4:2:2:2:1:1:1:1			32
- Lap1		4:2:2:2:2:1:1:1:1			32
- Mpi		4:2:2:2:2:1:1:1:1			32
- Pgd1		4:2:2:2:1:1:1:1			32
- Pgm1		4:2:2:2:1:1:1:1	126		32
 Dia3 - Idh1		9:3:3:1	60		31a
- Idh2		9:3:3:1	60		31a
- Idh3		6:3:3:2:1:1	171		32
- Mpi		6:3:3:2:1:1	148		32
- Pgd1		6:3:3:2:1:1	156		32
d1/d2- g		45:15:3:1			69
- i	(,7)		I		69
- r	(,2)		I3 independent		69
- t	(,1)		I genes		69
- w1	(,8)	45:15:3:1	I		69

Locus-to-locus linkage assays (continued)

Loci		Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
d1	- d2 - g	(3,) (3,3)	'independent' 6:4:3:2:1	no data shown 2462	4%	101 70
df2	- y11	(6,6)	6:3:2:1	1909	12%	105
df3	- df2	(,6)	9:3:3:1	95		9
df4	- df2 - df3	(,6)	9:3:4 9:3:4	150 164		29 29
df5	- y12 - t	(1,1) (1,1)	9:3:3:1 9:3:3:1	2195 2203	0% 14%	73 73
 df6	- df2 - df3 - df4 - df5	(,6)	6:3:3:4 6:3:3:4 6:3:7 6:3:3:4	192 189 202 98		107 107 107 107
dt1	- dt2 - 11	(5,) (5,5)	11:4:1 9:3:3:1	336 1474	39%	5 105
e1	- ÿ12 - e2 - e3	(1,1) (1,) (1,)	9:3:3:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1	1980 336 97	21%	102 6 15
e4	- fg1		9:3:3:1	81		12
e5	- e1 - e2 - e3	(,1)	other other other	188 227 111		63 63
Enp	- Idh2 - Pgm1 - Sod1 - Sp1 - t - w1	(,1) (,1) (,8)	4:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1	230 376 247 203 320+ 319+ 239* 193*		85 35 85 85 35 35 35
 Ep	- Sod1 - Sod2 - Sp1 - Ti	(12,) (12,) (12,1) (12,9)	9:3:3:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1	346* 345* 232 232		36 36 67 67

Locus-to-locus linkage assays (continued)

Loci		Linkage ¹ group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
f	- 1n - p1 - p2 - rj2 - rj4 - t - y3 - y9	(,4) (,2) (,4)	9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1	270 336 514 429 407 656 656 504		27 27 27 27 27 27 27 27 27
 fr1	- y10 - fr2 - fr3	(12,) (12,)	9:3:3:1 	456 		- 27 - 24 24 24
	- fr4 - p2 	(12,) (12,4)	9:7 9:3:3:1	104 595		24 27
fr4 fr5	- fr2 - fr1 - fr2 - fr3 - fr4	(,12)	9:7 9:7 9:7 9:7 9:7	97 377 364 334 343		- 24 - 81 81 81 81
	- t - w1 - y9 - y11 - y12	(,1) (,8) (,6) (,1)	9:3:3:1 9:3:3:1 9:3:3:1 6:3:3:2:1:1 9:3:3:1	484 1919 481 300 465		96 81 96 96 96
 fs1	- y13 - y20k2 - fs2	(,7)	9:3:3:1 9:3:3:1 —————————————————————————————————	485 561 		96 109
fs1/	/fs2 - st2 - st3 - st4 - t	(,1) (,8)	51:13 51:13 51:13 45:15:3 45:15:3	3076 1560 2707 1461 1196		109 109 109 109 109
g	- i - r - t - w1	(3,7) (3,2) (3,1) (3,8)	39:13:9:3 39:13:9:3 39:13:9:3 39:13:9:3	no data shown no data shown no data shown no data shown		69 69 69
Got	- Ap - Dia1 - Dia2 - Idh1 - Pgd2 - w1	(,9) (,9) (,8)	4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1	543 541 182 188 192 298		49 49 49 49 49 49

Locus-to-locus linkage assays (continued)

Loci	Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
Gpd - Idh2 - Idh3 - Lap1 - Mpi - Pgd1 - Pgm1		9:3:3:1 6:3:3:2:1:1 6:3:3:2:1:1 9:3:3:1 9:3:3:1 9:3:3:1	301 306 129 484 380 371		32 32 32 26 26 26
hs2 - hs1		9:7	174		20
hs3 - hs1 - hs2		9:7 9:7	173 173		20 20
i - r - t - w1 - y13	(7,2) (7,1) (7,8) (7,7)	39:13:9:3 9:3:4 39:13:9:3 9:3:3:1	no data shown no data shown no data shown no data shown	41%	69 69 69 69
Idh1 - Lap1 - Mpi - Pgd1 - Pgm1 - Pgm1 - Pgm2 - t - w1	(,9) (,1) (,8)	4:2:2:2:1:1:1:1 9:3:3:1 6:3:3:2:1:1 9:3:3:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1	721 350 195 108 280 595 706 131 412 203		51 32 32 32 32 32 32 88 51 31a 51 88
Idh2 - Idh1 - Lap1 - Mpi - Pgm1 - t	(,9)	4:2:2:2:2:1:1:1:1 8:3:3:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1	207 426 725 245 156 657 412		88 53 51 32 51 88 51
Idh3 - Idh1 - Idh2 - Lap1 - Mpi - Pgd1 - Pgm1		6:3:3:2:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1	119 119 296 213 201 312		53 53 32 32 32 32 32

Locus-to-locus linkage assays (continued)

Loci		Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
12	- Dia2		6:3:3:2:1:1	301		51
	- Idhl		6:3:3:2:1:1	319		51
	- Idh2		6:3:3:2:1:1	307		51
	- Lapl	(,9)	6:3:3:2:1:1	301		51
	- Pgml		6:3:3:2:1:1	262		51
	- Sp1	(,1)	6:3:3:2:1:1	322		51
Lap1	- Ap	(9,9)	4:2:2:2:1:1:1:1	3042	23%	21
			4:2:2:2:1:1:1:1	880	20%	54
			4:2:2:2:1:1:1:1	1154	24%	45
	<i>⊶ Dia2</i>	(9,)	4:2:2:2:1:1:1:1	268		51
	- Mpi		4:2:2:2:1:1:1:1	113		32
	- Pgml	(9,)	4:2:2:2:1:1:1:1	240		54
			4:2:2:2:1:1:1:1	655		54
	- Sp1	(9,1)	4:2:2:2:1:1:1:1	1430		54
			9:3:3:1	936	32	
	- t	(9,1)	6:3:3:2:1:1	412		51
	- Ti	(9,9)	4:2:2:2:1:1:1:1	2115	17%	21
			4:2:2:2:1:1:1:1	903	15%	48
			4:2:2:2:1:1:1:1	869	16%	45
Lap2	 ?- Dial		6:3:3:2:1:1	118		52
	- Lap1	(,9)	6:3:3:2:1:1	398		52
	- Pgm1	***	6:3:3:2:1:1	117		52
	- Sp1	(,1)	6:3:3:2:1:1	236		52
	- w1	(,8)	9:3:3:1	590		52
 &b1	- lb2	I				
	- t	(,1) I I	45:15:3:1	474		92
ℓ <i>b2</i>	- t	(,1)I				
	- lf2		4:2:2:2:1:1:1:1	116		 29
	v1	(4,4)	9:3:3:1	3596	35%	103
~	- p2	(4,4)	9:3:3:1	3223	26%	103
	P-2	(' ' ' ' /	9:3:3:1	297	22%	27
			9:3:3:1	439	27%	27
	- rj4	(4,)	9:3:3:1	329	- 7 70	27
	- w1	(4,)	9:3:3:1	297		27
	- y9	(4,)	9:3:3:1	380		27
	- y13	(4,7)	9:3:3:1	297		27
	 lw2		'independent'	no data shown		 91
	- t	(,1)	12:3:1	320		91
	- Le		9:3:3:1	336		43
	- Sp1	(,1)	9:3:3:1	254		43
	-	(,8)	9:3:3:1	334		43

Locus-to-locus linkage assays (continued)

Loci		Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
Lx2	- Lx1	(#)	9:3:3:1	74	?%	22
Lx3	- Lx1 - Lx2	I I	independent by m	node1		43
Mpi	- Pgd1 - Pgm1		9:3:3:1 9:3:3:1	389 372		26 26
ms2	- ms1 - y10 - y13	(,8) (,7)	3:1, 9:7 9:3:3:1 9:3:3:1	1873, 2093 163 256		33 65 65
ms3	- ms1 - ms2	(,8)	3:1, 9:7 3:1, 9:7	1384, 1598 673, 556		79 79
ms4	- ms1 - ms2 - ms3	(,8)	3:1, 9:7 3:1, 9:7 3:1, 9:7	997, 780 1233, 1596 1747, 1770		25 25 25 25
0	- i - y13	(7,7) (7,7)	6:3:3:2:1:1 9:3:3:1	3000 2001	18% 30%	105 105
p1	- Gpd - i - 12 - Mpi - o - p2 - pc - pd - Pgd1 - Pgm1 - ps - rj2 - t - w1 - y3 - y10 - y13	(2,) (2,7) (2,) (2,7) (2,4) (2,) (2,) (2,) (2,) (2,) (2,) (2,) (2,	9:3:3:1 9:3:3:1 'independent' 9:3:3:1 'independent' 12:3:1 12:1:2:1 12:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1	378 269 no data shown 491 no data shown 311 425 475 325 375 409 251 421 no data shown 672 595 1074 672		26 8 8 26 8 8 8 26 26 26 8 8 27 27 27 27
p2	- 0 - t - v1 - y9 - y10 - y13	(4,7) (4,1) (4,4) (4,) (4,) (4,7)	'independent' 'independent' 9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1	no data shown no data shown 2857 351 705 417		8 8 103 27 27 27

Locus-to-locus linkage assays (continued)

Loci		Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
pal	- p1 - pa2 - pc - pd - pi - ps	(,2)	'independent' 'independent' 'independent' 'independent' 'independent' 'independent'	no data shown		8 8 8 8 8
pa2	- p1 - pc - pd - pi - ps	(,2)	'independent' 'independent' 'independent' 'independent' 'independent'	no data shown no data shown no data shown no data shown no data shown		8 8 8 8 8
pc	- i - l2 - p2 - pd - ps - r	(,7) (,4) (,2) (,1)	'independent' 'independent' 9:3:4 6:3:3:2:1:1 6:3:3:2:1:1 'independent' 'independent'	no data shown no data shown 333 326 212 no data shown no data shown		8 8 8 8 8 8
pd	- i - p2 - ps - r - t	(,7) (,4) (,2) (,1)	'independent' 9:3:4 5:4:3:3:1 4:2:2:2:2:1:1:1:1 'independent' 'independent'	no data shown 331 348 372 no data shown no data shown		8 8 8 8 8
 Pgd1	- Ap - Lap1 - Pgi - Pgm1 - Ti	(,9) (,9) (#)	4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1:1 9:3:3:1 9:3:3:1 4:2:2:2:2:1:1:1:1	362 362 172 267 362	21%	21 21 32 21 21
Pgd2	- Ap - Lap1 - Pgd1 - Idh1 - Ti - w1	(9,9) (9,9) (9,) (9,) (9,9) (9,8)	4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1:1 6:3:3:2:1:1	2342 1064 406 188 1055	40% 21%	21 21 21 21 21 21 49
	- Ap - Lap1	(,9) (,9)	4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1	720 386		21 21
Pgm1 — — - ps	- t - i - p2 - t - w1	(,1) (,7) (,4) (,1) (,8)	6:3:3:2:1:1 'independent' 9:3:4 'independent' 'independent'	no data shown 340 no data shown no data shown		49 8 8 8 8 8

Locus-to-locus linkage assays (continued)

Loci		Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref
г	- p1	(2,2)	9:3:3:1 9:3:3:1		18%	103
		(2.7)	9:3:3:1	1575I 375		103
	- o - t	(2,7)	12:3:1 9:3:3:1	no data shown		69
	- L - w1	(2,1) (2,8)	9:3:3:1	no data shown		69
 rdc2	 - rdc1		15:1	340		 58
— — - rah2	 - rgh1	T	·			
-	- rgh1	I	7:1, 63:1	139, 1579		19
2	- rgh2	I				
rhg4	 - rgh1	(7,)	'independent'	no data shown		19
-	- rgh2	(7,)	'independent'	no data shown		19
	- rgh3	(7,)	'independent'	no data shown		19
	- i	(7,7)	'linked'	no data shown	35%	62
 rj1	 - y6		9:3:3:1	431		27
	- y9		9:3:3:1	322		27
	- y10		9:3:3:1	499		27
	- y13	(,7)	9:3:3:1	533	•	27
— — - rj2	 - t	(,1)	9:3:3:1	429		27
	- w1	(,8)	9:3:3:1	429		27
 rj4	 - Gpd		9:3:3:1	318		26
_	- Mpi		9:3:3:1	431		26
	- Pgd1 .		9:3:3:1	325		26
	- Pgm1		9:3:3:1	326		26
	- w1	(,8)	9:3:3:1	407		27
	- y10 		9:3:3:1	234		27
rj?	- Aco4		6:3:3:2:1:1	101		34
	- Dial		6:3:3:2:1:1	99		34
	- Sod1		9:3:3:1	101		34
	- Sp1	(,1)	6:3:3:2:1:1	101		34
	- t 	(,1)	9:3:3:1	101 		34
rmd	- fg3	(1,1)	9:3:3:1	120	I	14
	- t	(1,1)	9:3:3:1	673	I	14
	- y12 	(1,1)	9:3:3:1	674	_ I	14
rn2	- rn1		13:3	99		42
rpp2	- rpp1		12:1:2:1	176		40
 rpp3	- rpp1		10:5:1	188		40
	- rpp2		12:3:1	172		40

Locus-to-locus linkage assays (continued)

Loci	Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
rpp4 - rpp1 - rpp2 - rpp3		15:1 15:1 15:1	89 48 74		39 39 39
rps1 - Aco4 - Dia1 - dt1 - du - hm	(10,) (10,) (10,5) (10,) (10,10)	6:3:3:2:1:1 6:3:3:2:1:1 'independent' 'independent' 9:3:3:1 9:3:3:1 9:3:3:1	242 480 no data shown no data shown 648 290 624 300	22% 5% 6% 5%	84 83 38 38 57 57 56 56
- i - Idh1 - Idh2 - k1 - k2 - %1 - Pgm1 - r - t	(10,7) (10,) (10,) (10,) (10,) (10,) (10,) (10,2) (10,1) (10,8)	'independent' 6:3:3:2:1:1 6:3:3:2:1:1 'independent' 'independent' 6:3:3:2:1:1 'independent' 'independent' 'independent'	no data shown 227 227 no data shown no data shown 164 no data shown no data shown no data shown no data shown		38 83 38 38 38 38 38 38
rps2 - rps1	(,10)	15:1	1300		55
rps3 - Aco4 - Idh1 - Pgm1 - rps1	(,10)	6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 15:1	181 222 256 200		83 83 83 64
rps4 - Aco4 - Idh1 - rps1 - rps2 - rps3	(,10)	6:3:3:2:1:1 6:3:3:2:1:1 15:1 15:1 15:1	189 216 200 240 200		83 83 2 55 2
rps5 - Dia1 - Idh1 - Pgm1 - rps1 - rps2 - rps3 - rps4	(,10)	6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 15:1 15:1 15:1 15:1	209 463 216 116 240 59 72		83 83 83 14 55 15
rps6 - Aco4 - Dial - Enp - Idh1 - Idh2 - Pgm1		6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1	202 230 224 227 227 394		84 83 85 83 83 83

Locus-to-locus linkage assays (continued)

Loci	Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
rps6 - rps1 - rps2 - rps3 - rps4 - rps5	(,10)	15:1 15:1 15:1 15:1 15:1	200 120 200 240 110		1 55 1 1
rps? - Idh1 - Pgm1 - rps1 - rps2 - rps3 - rps4 - rps5	(,10)	6:3:3:2:1:1 6:3:3:2:1:1 other other other 15:1 15:1	243 238 100 261 289		83 86 86 86 86 86
rpv1 - rpv2 - t	(13,) (13,1)	13:3 45:15:3:1	445 296		98 10
rsv1 - rpv1 - rsv2 - t	(13,13) (13,) (13,1)	9:3:3:1 15:1 9:3:3:1	587 202 600	4%	89 18 27
se - dt1 - s - w1	(,5) (,8)	9:3:3:1 9:3:3:1 9:3:3:1	198 426 416		101 101 101
Sod1 - Aco1 - Aco2 - Aco4 - Ap - Dia1 - df2 - df4 - Idh1 - Idh2 - pb - Sp1 - t - w1 - y11	(,9) (,6) (,1) (,1) (,8) (,6)	6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 9:3:3:1 9:3:3:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 9:3:3:1 9:3:3:1	277+ 280+ 320+ 231* 348* 224 317 120 225* 349* 295+ 335* 349* 439		36 36 36 36 36 36 36 36 36 36 36 36 36
Sod2 - Aco3 - Aco4 - Ap - Dial - Idh2 - pb - Sod1 - Sp1 - t - w1	(,9) (,1) (,1) (,8)	4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1	152 ⁺ 314 ⁺ 282 313 314 348* 295 ⁺ 295 ⁺ 335* 348*		36 36 36 36 36 36 36 36 36

Locus-to-locus linkage assays (continued)

Loci		Linkage group	l Ratio evaluated	Population ² size	Map ³ distance	Ref.
Sp1	- Ap - Dial - Dia2 - dt1 - Gpd - Idh1 - Idh2 - Pgm1 - Pgm2 - Mpi - t	(1,9) (1,) (1,) (1,5) (1,) (1,) (1,) (1,) (1,) (1,1) (1,8) (1,1)	4:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 4:2:2:2:2:1:1:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1 6:3:3:2:1:1	117 404 125 232 310 378 426 445 415 111 175 708 1186 960 232 996 960	32% 31% 30% 22% 13%	32 32 67 32 32 51 51 32 32 51 37 46
 st4	- st2 - st3 - t - w1	(,1) (,8)	3:1, 9:7 3:1, 9:7 'independent' 'independent'	1577, 1296 1178, 1171 no data shown no data shown		78 78 78 80 80
st5	- st2 - st3 - st4 - t	(8,) (8,) (8,) (8,1) (8,8)	3:1, 9:7 3:1, 9:7 3:1, 9:7 9:3:3:1 9:3:3:1 9:3:3:1	1531, 362 1523, 795 784, 859 1584 1239 499	27% 27%	74 74 74 74 74 74
Sun	– – – – – – Eu	(14,14)	5:5:4:2	550	1%	60
t t	- d1 - d2 - e1 - e2 - g - k1 - y3 - y12	(1,3) f (1,) (1,1) (1,3) (1,) (1,) (1,1)	nformation for d1 d2 on 6:3:3:2:1:1 9:3:3:1 4:2:2:2:2:1:1:1:1 9:3:3:1 'independent' 9:3:3:1 9:3:3:1 9:3:3:1	621 1980 351 161 no data shown 656 4030 960	4% 22% 22%	102 102 102 102 102 69 98 27 102 46
td	- i - r - t	(,7) (,2) (,1)	'no sign close linkage' 'no sign close linkage' 9:3:4			4 4 4
 Ti	- dt1	(,5)	6:3:3:2:1:1	232		 67
— — wm	- ms1	(8,8)	other	310		94

Locus-to-locus linkage assays (continued)

Loci		Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
w1	- k1 - rps3	(8,) (8,)	4:2:2:2:1:1	600 280		98 87
	- rps4 - rps5 - rps? - w3	(8,) (8,) (8,) (8,)I	9:3:3:1 9:3:3:1 9:3:3:1	256 320 255		87 87 87
	- w4	(8,)I	36:9:19	992		38
	- y9 - y13	(8,) (8,7)	9:3:3:1 9:3:3:1	298 672		27 27
w3	- w4		12:3:1	1329		38
y 3	- g	(,3)	'independent'	no data shown		95 — 9
y5	- y4 - y6 - y9 - y10		9:7 9:7 9:7 9:7	63 140 30 57		66 66 66
	- y12 - y13 - y14	(,1) (,7)	9:7 9:7 9:7	49 41 23		66 66 66
	- y15 - y17		9:7 9:7	29 46		66 66
— — · у9	- f - ln - w1 - y11	(,11) (,4) (,8) (,6)	9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1	no data shown no data shown no data shown no data shown		82 82 82 82 7
— — - y17	- y4 - y6 - y10 - y12 - y13 - y14 - y15	(,1) (,7)	9:7 9:7 9:7 9:7 9:7 9:7 9:7	30 23 44 39 28 53 47		66 66 66 66 66 66
у18	- w1	(,8)	9:3:3:1	225		97
y20k	2- Ep - fr1 - pb - %1 - gy3 - y5 - y9 - y10	(,12) (,12) (,5)	9:3:3:1 9:3:3:1 9:3:3:1 9:3:3:1 70.3:29.7 9:7 9:3:3:1 9:7	699 699 699 212 1003 556 883 3221		72 72 72 72 72 72 72 72 72 72

Locus-to-locus linkage assays (continued)

Loci	Linkage ^l group	Ratio evaluated	Population ² size	Map ³ distance	Ref.
y20k2- y11	(,6)	3:13	1108		72
- y12	(,1)	9:7	1129		72
- y13	(,7)	9:7	833		72
- y18		9:7	981		72

¹The linkage group for each locus appears in parentheses. If there is no number on one side, or the other, of the comma, linkage group is unknown.

References

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 $^{^2\}mathrm{Refers}$ to F_2 population size of largest population assayed, or, if appropriate, of combined F_2 populations.

Map distances are approximate only.

 $^{^{+}}$ Indicates that F $_{3}$ data confirm the F $_{2}$ data.

^{*}Indicates that only F_3 data were used.

[#]Indicates that no official linkage group designation has been given to this pair of loci, although they are apparently linked.

Independent loci within a single linkage group.

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XI. APPROVED SOYBEAN GENE SYMBOLS

Soybean scientists established a Soybean Genetics Committee in 1955. Its functions include: 1) maintain a Genetic Type Collection, 2) establish guidelines and rules for assigning gene symbols, and 3) act as a review committee for manuscripts concerning qualitative genetic interpretation and gene symbols in the genus *Glycine*. At its annual meeting in February 1987, the Committee decided that a complete list of approved soybean gene symbols should be published in the Soybean Genetics Newsletter in 1987, and every 5 years thereafter. A cumulative update of new symbols should be published between the full updates.

Table 1 includes the bulk of all gene symbols approved in soybean, with the exception of the most recently accepted and named, which appear in Table 2. Several listings inadvertently omitted from Table 1 are included in Table 2. Each table has its own reference list; the numbers in the Reference column refer only to the references accompanying that particular table.

Table 1. Genetic loci of soybean

Gene	Strain	Phenotype	Reference
Ab	T161, c*	Leaf abscission at maturity	144
ab	Kingwa	Delayed abscission	
Acol-a	Evans, Weber	Aconitate hydratase mobility variant	55,69
Acol-b	PI 257430**	Aconitate hydratase mobility variant	
Aco2-a	PI 464918	Aconitate hydratase mobility variant	69
Aco2-b	Evans, Weber	Aconitate hydratase mobility variant	
Aco3-a	Amsoy 71, Weber, Evans, T161	Aconitate hydratase mobility variant	69
Aco3-b	PI 342622A (wild soybean), PI 437728, PI 438463	Aconitate hydratase mobility variant	
Aco4-a	Williams	Aconitate hydratase mobility variant	69
Aco4-b	Evans	Aconitate hydratase mobility variant	
Aco4-c	Minsoy	Aconitate hydratase mobility variant	
Adhl adhl	Altona, Wilson A-100, Lindarin	Alcohol dehydrogenase present Alcohol dehydrogenase absent	62,88,90
Adh2 adh2	Amsoy, Beeson Cayuga, Grant	Alcohol dehydrogenase present Alcohol dehydrogenase absent	62,88
Amyl amyl	Harosoy, Clark Altona, PI 132201	lpha-amylase band l present $lpha$ -amylase band l absent	61,62,85
Amy2 amy2 Amy3	Harosoy, Clark Altona, PI 132201	α -amylase band 2 present α -amylase band 2 absent (See $Sp1$)	61,62,85
Ap-a	Ebony	Acid phosphatase mobility variant	46,61,80
Ap-b	Amsoy 71	Acid phosphatase mobility variant	86,89,94
Ap-c	Earlyana, Manchu	Acid phosphatase mobility variant	
B1 B2 B3	Sooty	Bloom on seed coat	168,192
b1, b2,	С	No bloom	193
or b3			
Cgyl	С	β -conglycinin subunit $lpha'$ present	103
Cgyl-s	PI 54608-1	$\beta\text{-conglycinin}$ subunit α^{I} present mobility variant	49
cgy1	Keburi	β -conglycinin subunit $lpha'$ absent	
Cgy2 or	Raiden	lpha subunit of eta -conglycinin produced	49,172
Cgy2-a			
Cgy2-S or	PI 54608-1	$_{lpha}$ subunit of $_{eta}$ -conglycinin produced, exhibits slowed mobility	
Cgy2-b			
Cgy3-D	PI 81041-1	At lease one polypeptide of the β subunit of β -conglycinin exhibits	49,172
cgy3-D	PI 253651	increased mobility - (β') β' subunit of β -conglycinin not produced	
D1	С	Yellow seed embryo	126,174, 190
d1	Columbia, T104	Green seed embryo	1 70
Df2	C C C C C C C C C C C C C C C C C C C	Normal height	43,143
df2	T210,T243	Dwarf	.0,1.0

Table 1. (continued)

Gene	Strain	Phenotype	Reference
df3	T244	Dwarf	
Df4	С	Normal height	58
df4	T256	Dwarf	122 124
Df5 df5	с Т263	Normal height Dwarf	133,134
Dial-a	Evans, Elton	Diaphorase mobility variant	65,88
Dial-b	Cayuga, Kingston	Diaphorase mobility variant	
Dia2-a	Amsoy, Elton	Diaphorase mobility variant	65,88
Dia2-b	Wilson, Kingston	Diaphorase mobility variant	(F 00
Dia3 dia3	Kingston Elton	Diaphorase present Diaphorase absent	65,88
Dt1	Manchu, Clark	Indeterminate stem	9,182,192
dt 1	Ebony, PI 86024	Determinate stem	193
Dt2	T117	Semideterminate stem	9
dt2	Clark	Indeterminate stem	
E1	T175	Late flowering and maturity	8,127
e1	Clark	Early flowering and maturity	
E2	Clark	Late flowering and maturity	8
e2	PI 86024	Early flowering and maturity	22.00
E3	Harosoy 63	Late flowering and maturity; sensitive to fluorescent light	32,99
e3	Blackhawk	Early flowering and maturity;	
	Dideknawk	insensitive	
E4	Harcor	Sensitive to long day length	42
e4	Urozsajnaja (PI 297550)	Insensitive	
Enp-a	Evans, Weber	Endopeptidase mobility variant	69
Enp-b	PI 257430	Endopeptidase mobility variant	
Ep	Harosoy 63	High seed coat peroxidase activity	37,137
ep Eu	Blackhawk Blackhawk,	Low seed coat peroxidase activity Urease fast band	29
	Chippewa 64		29
eu F	Corosoy, Midwest	Urease slow band Normal stem	2 52 111
f	c T173, PI 83945-4	Fasciated stem	2,53,111 118,164,
1	11/3, 11 03943-4	rasciated stell	192,193
Fas	Coles	Normal level of stearic acid in seed	67
fas-a	A6 (sodium azide- induced mutant of FA 8077)	Elevated level of stearic acid in seed	
fas-b	AlO (EMS-induced mutant of Coles)	Elevated level of stearic acid in seed	
fas	A9 (EMS-induced mutant of FA 9886)	Elevated level of stearic acid in seed	67
Fe	c	Efficient iron utilization	178
fe	PI 54619	Inefficient	
Fg1	Т31, с	β (1-6)-glucoside present	31
fg1	Chippewa 64, c	β (1-6)-glucoside absent	
Fg2 fg2	T31, c Chippewa 64, c	α (1-6)-rhamnoside present α (1-6)-rhamnoside absent	31

Table 1. (continued)

Gene	Strain	Phenotype	Reference
Fg3	T31	β (1-2)-glucoside present	31,33,34
fg3	Chippewa 64	β (1-2)-glucoside absent	35,40
Fg4	T31	α (1-2)-rhamnoside present	31,33,34
fg4	AK (FC 30761)	α (1-2)-rhamnoside absent	35,40
Fr1	С	Roots fluorescent in UV light	60,137
fr1	Minsoy (PI 27890)	Roots nonfluorescent in UV light	
Fr2	С	Roots fluorescent in UV light	51
fr2	Noir-1 (PI 290136)	Roots nonfluorescent in UV light	
Fr3	PI 424078	Roots nonfluorescent in UV light	51
fr3	С	Roots fluorescent in UV light	- 1
Fr4	C	Roots fluorescent in UV light	51
fr4	Dun-cuan (PI 404165)	Roots nonfluorescent in UV light	
Fr5	С	Roots fluorescent in UV light	130,152
fr5	T285	Roots nonfluorescent in UV light	
Fs1 or	С	Fertile	83
Fs2	T260	2	
fsl fs2	T269	Structural sterile	156
Ft	C	Fertile	156
ft	Gamma ray-induced mutant	Structural sterile	
G	Kura	Green seed coat	117,167, 169,190
g	С	Yellow seed coat	
Got-a	PI 407194	Glutamate oxaloacetic transaminase	92
	(wild soybean)	mobility variant	
Got-b	PI 407221 (wild	Glutamate oxaloacetic transaminase	
	soybean), PI 407160 (wild	mobility variant	
	soybean)		
Got-c	PI 407207	Glutamate oxaloacetic transaminase	
. 1	(wild soybean)	mobility variant	65 00
Gpd	Amsoy, Evans	Glucose-6-phosphate dehydrogenase present	65,88
gpd	Chestnut, Cayuga	Glucose-6-phosphate dehydrogenase (wea	
Gy4	С	Glycinin subunit A5A4B3 present	.103
gy4	Raiden	Glycinin subunit A5A4B3 absent	2.3
Нb	Clark 63	Tolerant to the herbicide bentazon	21
hb	Nookishirohana (PI 229342)	Sensitive	
Hm	Hood	Tolerant to the herbicide metribuzin	56,98
hm	Semmes	Sensitive	
I	Mandarin	Light hilum	109,117, 119
i	T152, T157, Soysota	Self dark seed coat	128,192, 193
i-k	Black Eyebrow	Saddle pattern on seed coat	193
1-K 1-1	Manchu	Dark hilum	
Idh1-a	Amsoy, Cayuga	Isocitrate dehydrogenase mobility	65,88,
Julia d		variant	195,196

Table 1. (continued)

Gene	Strain	Phenotype	Reference
Idh1-b	Wilson, Evans	Isocitrate dehydrogenase mobility variant	
Idh2-a	Amsoy, Cayuga	Isocitrate dehydrogenase mobility variant	65,88, 195,196
Idh2-b	Wilson, Evans	Isocitrate dehydrogenase mobility variant	,
Idh3-a	Elton, Amsoy	Isocitrate dehydrogenase mobility variant	65,88, 195,196
Idh3-b	Agate, Wilson	Isocitrate dehydrogenase mobility variant	
Im	Merit	Nonmottled seed	47
im	Harosoy	Dark mottled seed	
K1	С	Nonsaddle	165,165
k1	Kura, T153, Agate	Dark saddle on seed coat	188
K2	С	Yellow seed coat	135,148
k2	T239, T253, T261	Tan saddle on seed coat	
K3	С	Nonsaddle	22
k3	T238	Dark saddle on seed coat	
L1	Seneca	Black pod	7,182
L 1	Clark, Dunfield	Brown or tan pod,	
L2	Seneca, Clark	interacts with $L2/2$ Black or brown pod,	7
0.0	DT 05505 D 6: 11	interacts with L1/L1	
l2	PI 85505, Dunfield	Black or tan pod, interacts with $L1/21$	
Lap1-a	Norredo, Wilson	Leucine aminopeptidase mobility variant	
Lap1-b	Lindarin	Leucine aminopeptidase mobility variant	
Lap2-a	PI 163453 (wild soybean)	Leucine aminopeptidase mobility variant	-
Lap2-b	Amsoy	Leucine aminopeptidase mobility variant	
lap2	Jefferson	Leucine aminopeptidase absent	
Lb1 $Lb2$	Harosoy	Nonbullate leaf	150
Lb1 lb2	Clark	Nonbullate leaf	
ℓ <i>b1 Lb2</i>	T217	Nonbullate leaf	
lb1 lb2	L65-701 (Clark ⁶ x PI 19616)	Bullate leaf	
Le	Harosoy	Seed lectin present	125,146
le	T102	Seed lectin absent	159
Lf1	PI 86024	5-foliolate leaf	59,167
&f1	С	Trifoliolate leaf	
Lf2	C	Trifoliolate leaf	59
ℓ <i>f2</i>	T255	7-foliolate leaf	00 51 11
Ln	C	Ovate leaflet	22,54,166
l n	T41, PI 84631	Narrow leaflet, 4-seeded pods	167,181
Lo	c T122	Ovate leaflet for gooded reds	54
lo	Lee 68	Oval leaflet, few-seeded pods	97
Lps	T279	Normal petiole Short petiole	71
lps Lw1 Lw2	12/9	Nonwavy leaf	149
TMT TMS		nonwavy ital	147

Table 1. (continued)

Gene	Strain	Phenotype	References
Lw1 lw2	Harosoy, Clark	Nonwavy leaf	
lw1 Lw2	T117	Nonwavy leaf	
lw1 lw2	T176, T205	Wavy leaf	
Lx1	Harosoy, Clark	Lipoxygenase-l present	6,79,104
lx1	Kedelee No. 367, PI 408251	Lipoxygenase-l absent	
Lx2	Suzuyutaka	Lipoxygenase-2 present	49,104,10
lx2	PI 86023	Lipoxygenase-2 absent	
Lx3	Raiden, Century	Lipoxygenase-3 present	102
lx3	Wase Natsu (PI 417458), I-Higo-Wase, (PI 205085)	Lipoxygenase-3 absent	
Mn	С	Normal plant	52
mn	T251	Miniature plant	
Mpi-a	Wilson, PI 65549 (Wild soybean)	Mannose-6-phosphate isomerase mobility variant	65,88
Mpi-b	Amsoy, Kingston	Mannose-6-phosphate isomerase mobility variant	
Mpi-c	Elton, Hark	Mannose-6-phosphate isomerase mobility variant	
Ms1	С	Fertile	23,27,131, 136,140
ms-1	(North Carolina) T260H	Male sterile	27
ms-1	(Urbana) T266H	Male sterile, higher female fertility	23
ms T	(UIDalla) 120011	than other ms1 isolates	23
ms1	(Tonica) T267H	Male sterile	140
ms1	(Ames 1) T268H	Male sterile	136,140
Ms2	С	Fertile	16,68
ms2	T259	Male sterile	. , .
Ms3	С	Fertile	138
ms3	T273	Male sterile	
Ms4	C	Fertile	50
ms4	T274	Male sterile	
Ms5	C	Fertile	28
ms5	T277	Male sterile	160 161
Msp	C m 2.7.1	Fertile	160,161
msp N	T271	Partial male sterile Normal hilum abscission	128
	c Soysota	Lack of abscission layer	120
n Nc1	Lee	Chloride-excluding	1
nc1	Jackson	Chloride-accumulating	1
Np	Chief	Phosphorus-tolerant	18
np	Lincoln	Sensitive to high P-level	
Nr	Williams	Constitutive nitrate reductase present	151
nr	T276	Constitutive nitrate reductase absent	
0	Soysota	Brown seed	117,180
0	Ogemaw	Reddish brown seed	
P1	T145	Glabrous	119

Table 1. (continued)

Gene	Strain	Phenotype	Reference
p1	С	Pubescent	
P2	С	Normal	163,181
p2	T131	Puberulent	
Pal Pa2	Harosoy, Clark	Erect pubescence	13,84,171
Pa1 pa2	(Harosoy ^b x Higan)	Erect pubescence	
pal Pa2	Scott, Custer, Oksoy	Semi-appressed pubescence	
pa1 pa2	Higan	Appressed pubescence	
Pb	PI 163453 (wild soybean), Kingwa	Sharp hair tip	171
pb	Clark	Blunt hair tip	
PC	Clark, c	Normal pubescence	19
pc	PI 84987	Curly (deciduous) pubescence	
Pd1	PI 80837	Dense pubescence	19
pd1	Clark, c	Normal pubescence	1./
Pd2	T264	Dense pubescence	14
pd2 Pgd1 - a	Clark, c Agate, Kingston	Normal pubescence Phosphogluconate dehydrogenase mobility variant	65,88
Pgd1-b	Elton, Hill	Phosphogluconate dehydrogenase mobility variant	
Pgd1-c	PI 407160 (wild	Phosphogluconate dehydrogenase mobility	
1941 0	soybean)	variant	
pgd	Hidaka-1 (PI 406684), PI 65549 (wild soybean)	Phosphogluconate dehydrogenase absent	
Pgd2-a	PI 487428, AV62, PI 424032 (wild soybean), PI 40722 (wild soybean)	6-phosphogluconate dehydrogenase mobility variant 3	46,65,88
Pgd2 - b	PI 407192 (wild soybean), PI 407160 (wild soybean)	6-phosphogluconate dehydrogenase mobility variant	
Pgd2-c	PI 404252	6-phosphogluconate dehydrogenase mobility variant	
Pgd3-a	PI 486220 (wild soybean)	Phosphogluconate dehydrogenase mobility variant	46
Pgd3-b	PI 487431 (wild soybean)	Phosphogluconate dehydrogenase mobility variant	
Pgi-a	PI 135624 (wild soybean) PI 65549 (wild soybean)	Phosphoglucose isomerase mobility variant	65,88
Pgi-b	Beeson, Hark	Phosphoglucose isomerase mobility variant	
Pgml-a Pgml-b	Chestnut, Wells Amsoy, Hark	Phosphoglucomutase mobility variant Phosphoglucomutase mobility variant	65,88

Table 1. (continued)

Gene	Strain	Phenotype	Reference
Pgm2-a	PI 423990 (wild soybean), Shirosaya l (PI 423955)	Phosphoglucomutase mobility variant	
Pgm2-b Pm	Amsoy, Wells	Phosphoglucomutase mobility variant Normal	144
pm Ps Ps-s	T211 PI 91160 Higan	Dwarf, crinkled leaves, sterile Sparse pubescence Semi-sparse pubescence	12,19
ps	С	Normal pubescence	117 110
R r r-m Rcs1	c c T146, PI 91073 Lincoln, Wabash	Black seed Brown seed Black stripes on brown seed Resistant to frogeye leaf spot, race 1	117,119 162,180 187,190 3,145
rcs1	Gibson, Patoka, Hawkeye	Susceptible	
Rcs2 rcs2	Kent C1043 (PI 70237 x Lincoln), C1270 Mandarin (Ottawa) x Clark	Resistant to frogeye leaf spot, race 2 Susceptible	145
Rcv	Bragg, Hill, Lee	Resistant to cowpea chlorotic mottle virus	26
rcv	Davis, Hood, Jackson	Susceptible	
rhg1, rhg2, rhg3	Peking	Resistant to cyst nematode	45
Rhg1, Rhg2, Rhg3	Lee, Hill	Susceptible	
Rhg4 with rhg1, rhg2, rhg3	Peking	Resistant to cyst nematode	110
rhg4 Rjl	Scott T180, T202	Susceptible Nodulating	44,53,189
rj1 Rj2	T181, T201 Hardee, CNS	Nonnodulating Ineffective nodulation by strains b7, b14, and b122	44
rj2 Rj3	c Hardee	Effective	175
rj3 Rj4	Clark Hill, Dare,	Ineffective nodulation by strain 33 Effective Ineffective nodulation by strain 61	176
rj4 Rmd	Dunfield Lee, Semmes Blackhawk	Effective Resistant to powdery mildew	39
rmd Rpg1 rpg1	Harosoy 63 Norchief, Harosoy Flambeau	Susceptible Resistant to bacterial blight, race l Susceptible	115

Table 1. (continued)

Gene	Strain	Phenotype	Reference
Rpm	Kanrich	Resistant to downy mildew	15
rpm Rppl	Clark, Chippewa Komata (PI 200492)	Susceptible Resistant to soybean rust	112
rpp1 Rpp2	Wills, Davis PI 230970 c	Susceptible Resistant to soybean rust Susceptible	73
rpp2 Rpp3 rpp3	Ankur (PI 462312)	Resistant to soybean rust Susceptible	73
Rps1	Mukden	Resistant to phytophthora rot, races 1, 2, 10, 13, 16	20,75,105, 113
Rps1-b	FC 31745, Sanga, PI 84637	Resistant to phytophthora rot, races 1, 3-9, 13-15, 17, 18, 21, 22	75,91,108, 116
Rps1-c	Mack, PI 54615-1	Resistant to phytophthora rot, races 1-3, 6-11, 13, 15, 17, 21	108,116
Rps1-k	Kingwa	Resistant to phytophthora rot, races 1-10, 13-15, 17, 18, 21, 22	17,108
rps1	Lincoln, Harosoy	Susceptible	20,75,105, 113
Rps2	CNS	Resistant to phytophthora rot, races 1-2	101
rps2 Rps3	c PI 171442, PI 86972-1	Susceptible Resistant to phytophthora rot, races 1-5, 8, 9, 11, 13, 14, 16, 18	108, 116
rps3 Rps4	Harosoy PI 86050	Susceptible Resistant to phytophthora rot, races 1-4, 10, 12-16	5
rps4 Rps5	Harosoy PI 91160	Susceptible Resistant to phytophthora rot, races 1-5, 8, 9, 11, 13, 14, 16	36
rps5	Harosoy	Susceptible	
Rps6	Altona	Resistant to phytophthora rot, races 1-4, 10, 12, 14-16, 18-21	4,108
rps6 Rpv1	Harosoy Dorman, CNS	Susceptible Resistant to peanut mottle virus, M-2	25,147
rpv1 Rpv2	Bragg, Pickett PI 229315	Susceptible Susceptible to peanut mottle virus, PmV-S/V745	155
rpv2	Peking	Resistant	
Rrn	Ransom	Susceptible to reniform nematode	185
rrn	Forrest	Resistant	05 1/7
Rsv1	PI 96983	Resistant to soybean mosaic virus, SMV-1, SMV-1-B	95,147
rsv1	Hill Talana Ondan	Susceptible	
rsv1-t	Tokyo, Ogden	Resistant to SMV-1, susceptible to SMV-1-b	
Rsv2	Raiden	Resistant to soybean mosaic virus, G7, G7A	41
rsv2	Williams	Susceptible	
Rxp rxp	Lincoln, Ralsoy CNS	Susceptible to bacterial pustule Resistant	22,57,76

Table 1. (continued)

Gene	Strain	Phenotype	Reference
S	Higan	Short, internode length decreased	10
S	Harosoy	Normal internode length	
s-t	Chief	Tall, internode length increased	
Sbl or	Davis	Normal stem	24,96,100
Sb2	Davis	normal ocen	21,70,100
sb1 sb2	Ya Hazi	Brachytic stem	
	(PI 227224)		
Se	T208	Pedunculate inflorescence	173
se	PI 84631	Subsessile inflorescence	
Sod1	С	Superoxide dismutase bands 4 and 5 present	62,64,66, 70
sod1	Evans	Superoxide dismutase bands 4 and 5 absent	
Sod2-a	Polysoy	Superoxide dismutase mobility variant	69
5002-a 50d2-b	Evans, Williams 82	Superoxide dismutase mobility variant	57
	Amsoy, Evans	β-amylase mobility variant	61,62,69,
Sp1-a	Allooy, Evallo	p-amylase modificy variant	71,77,78,
a. 1 1	Hilliams Contury	β-amylase mobility variant	89
Sp1-b	Williams, Century	p-amyrase modificy variant	82,85,87,
	01	01	106,107
spl-an	Chestnut	β-amylase activity weak or absent,	122
		seed protein band present	
sp1	Altona***,	β-amylase activity absent, seed	
	PI 132201	protein band absent	
St2	С	Fertile	72
st2	T241	Asynaptic sterile	
St3	С	Fertile	72
st3	T242	Asynaptic sterile	
St4	C	Fertile	131
st4	T258	Desynaptic sterile	
St5	С	Fertile	139
st5	T272	Desynaptic sterile	
Γ	С	Tawny (brown) pubescence, quercetin	30,69,71,
		and kaempferol present	87,89,117
t	С	Gray pubescence, quercetin absent, kaempferol present	142,186,1
Td	Clark	Tawny pubescence (brown); flavonol	11,30
ra	Clair	present	11,50
	C	•	
td	Grant, Sooty	Light tawny pubescence (near gray); flavonol absent	
Ti-a	Harosoy, Clark	Kunitz trypsin inhibitor mobility variant	46,81,86, 123,124,1
Ti-b	Aoda	Kunitz trypsin inhibitor mobility variant	
Ti-c	PI 86084	Kunitz trypsin inhibitor mobility variant	
ti	Baik Tae	Kunitz trypsin inhibitor absent	
	(PI 196168)	Name 1 1 and address of the state of the sta	101 100 1
V1	C	Normal leaf pigmentation	181,192,1

Table 1. (continued)

Gene	Strain	Phenotype	Reference
v1	Т93	Variegated leaves	
W1	С	Purple flower	90,132,136 153,167, 191
w1	С	White flower	
W3 w4	Laredo	Dilute purple flower	74
w3 W4	c	Purple flower	
W3 W4	L70-4422 [L6 ⁶ x (Laredo x Harosoy)]	Dark purple flower	
w3 w4	L68-1774 [L6 ⁶ x (Laredo x Harosoy)]	Near white flower	
Wm	c	Purple flower, glycosides present	38,153
wm	T235	Magenta flower, glycosides absent	-
Y3	С	Normal foliar pigmentation	114,118, 164,165, 170
y3	Kura, T139	Green seedling, becoming yellow	
Y4	С	Normal foliar pigmentation	114,194
y4	T102	Greenish-yellow leaves, weak plant	
Y5	С	Normal foliar pigmentation	114,194
y5	T116, T134	Greenish-yellow leaves	
Y6	c	Normal foliar pigmentation	114,194
y6	T136	Pale green leaves	
Y7 or	С	Normal foliar pigmentation	114,144
Y8	T138	Volley arouth in seel weether	186
y7 y8 Y9	C C	Yellow growth in cool weather Normal foliar pigmentation	144
49	T135	Bright greenish-yellow leaves	144
y10	C C	Normal foliar pigmentation	144
y10	T161	Greenish-yellow seedling	144
Y11	c	Normal foliar pigmentation	177
u11	T219	Lethal yellow	177
Y12	c	Normal foliar pigmentation	71,87,179
y12	Т233	Whitish primary leaves, yellowish- green leaves	
Y13	С	Normal foliar pigmentation	183
y13	Т230	Whitish-green seedling, greenish- yellow leaves	
Y14	c	Normal foliar pigmentation	120
y14	T229	Light green leaves	
Y15	C	Normal foliar pigmentation	120
y15	T234	Pale yellowish-green leaves	10/
Y16	C m257	Normal foliar pigmentation	184
y16	T257	Nearly white lethal	1.0.1
Y17	C T162	Normal foliar pigmentation	121
y17	T162	Light yellowish-green leaves, normal foliar pigmentation	
Y18	С	Normal foliar pigmentation	141
110		Mormar rollar bigmentarion	141

Table 1. (continued)

Gene	Strain	Phenotype	Reference
y18-m	T225M	Unstable allele resulting in chloro- phyll chimera	
y18	T225H	Near-lethal yellow	154
Y19	С	Normal foliar pigmentation	158
y19	T265	Delayed albino	
Y20 K2	С	Normal foliar pigmentation	135
y20 k2	T253	Yellowish-green leaves, weak plant; tan saddle on yellow seed coat	

^{*}c indicates that the gene occurs in many cultivars.

**PI indicates Plant Introduction strains. Collection consists of foreign strains assigned identifying numbers by the Germplasm Resources Laboratory, Plant Genetics and Germplasm Institute, Agricultural Research Center - West, Beltsville, Maryland.

***Altona is a mixture of several genotypes.

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 $\mbox{\ensuremath{\mbox{\tiny *}Genetic}}$ symbols and (or) linkage interpretation approved by Soybean Genetics Committee.

Table 2. Recently named genes, plus some inadvertently omitted from Table 1.

Gene	Strain	Phenotype	Reference
Df6	С	Normal height	15
df6	EMS-induced	Dwarf	
	mutant of C1421		
E5	L64-4830	Late flowering and maturity	10
e5	Harosoy, Clark	Early flowering and maturity	
Eu-a	Columbia	Hexamere seed urease	8
Eu-b	Williams	Trimere seed urease	1.6
Fan	C	Normal level of linolenic acid in seed	16
fan	EMS-induced mutant of C1640	Low level of linolenic acid in seed	7
Hs1	Williams	Sensitivity to sulfonylurea herbicides	
hs1	EMS-induced mutant	Enhanced tolerance	12
Hs2	Williams	Sensitivity to sulfonylurea herbicides	12
hs2	EMS-induced mutant	Enhanced tolerance	1.0
Hs3	Williams	Sensitivity to sulfonylurea herbicides	12
hs3	EMS-induced mutant	Enhanced tolerance	1 /
ms1	(Ames 2) AP6(S1)C1	Male sterile	14
Rbs1 rbs1	L78-4094 LN78-2714	Resistant to brown stem rot	3
Rbs2	PI 437833	Susceptible Resistant to brown stem rot	3
rbs2	Century	Susceptible	3
Rdc1	Tracy	Resistant to stem canker	6,7
rdc1	J77-339	Susceptible	0,7
Rdc2	Tracy	Resistant to stem canker	6,7
rdc2	J77-339	Susceptible	0,7
Rn1	Bragg, Davis	Susceptible to reniform nematode	4
rn1	Dare, Pickett 71	Resistant	
Rn2	Dare, Davis	Susceptible to reniform nematode	4
rn2	Bragg, Pickett 71	Resistant	
Rps1-d	PI 103091	Resistant to phytophthora rot races 1-5, 7, 9, 10, 13-16, 18, 20-24	5
Rps3-b	PI 172901	Resistant to phytophthora rot races 1-5, 7, 9, 10, 12, 16	11
Rps3-c	PI 340046	Resistant to phytophthora rot races 1-4, 12, 16	1
Rps3-d	PI 82312N	Resistant to phytophthora rot races	5
Rps3-e	PI 273483D	Resistant to phytophthora rot races 1-4, 12, 14, 16	5
Rps7	PI 82312N	Resistant to phytophthora rot races 1-5, 7-9	5
rps7	Harosoy	Susceptible	5
Rym1	PI 171443	Resistant to yellow mosaic virus	13
rym1	Bragg	Susceptible	- 3
Rym2	PI 171443	Resistant to yellow mosaic virus	13
rym2	Bragg	Susceptible	_
Sp1-c	PI 464918	β-amylase mobility variant	2
Sun	С	Urease present	8
sun	PI 229324	Urease absent	
Y21	С	Normal	18
y21	Shennong 2015	Lethal yellow	

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1) Designation of a "core" collection of perennial Glycine.

Over the last decade, a large germplasm collection of the 12 currently recognized perennial species of *Glycine* has been assembled. This collection, now numbering more than 1400 accessions, is held in Canberra, Australia, and is recognized by the International Board of Plant Genetic Resources as the world base collection for perennial *Glycine*. The 12 species include five that have been described recently. These are *G. argyrea* and *G. cyrtoloba* (Tindale, 1984), *G. curvata* (Tindale, 1986a) and *G. microphylla* and *G. arenaria* (Tindale, 1986b).

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For many purposes, the large size of the whole collection makes it difficult to use. Furthermore, as with most germplasm collections, the accessions have been received in batches, often of several closely similar lines. To facilitate the conservation, exchange, research, and use of these genetic resources for soybean improvement, a "core" collection has now been defined. The general reasons and methods for setting up core collections are discussed in detail by Frankel and Brown (1983) and Brown (1987). The aim of the core collection is to represent with minimum size and repetitiveness, as broad a range of the genetic diversity in the collection as possible. The remaining accessions of the collection are not discarded, but retained as the "reserve" collection.

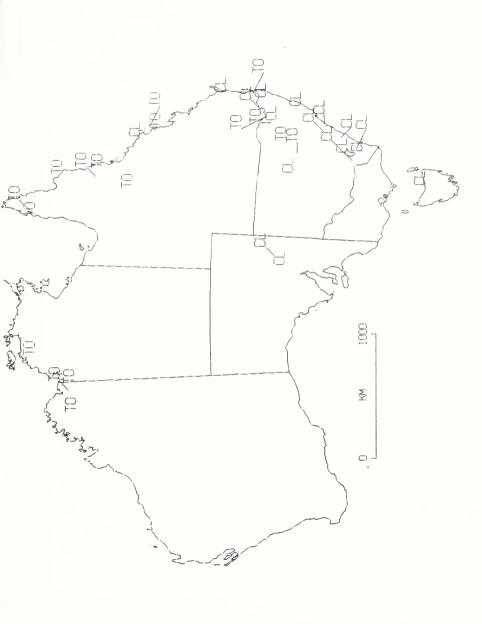
In the case of ${\it Glycine}$, the accessions forming the core collection were chosen as follows:

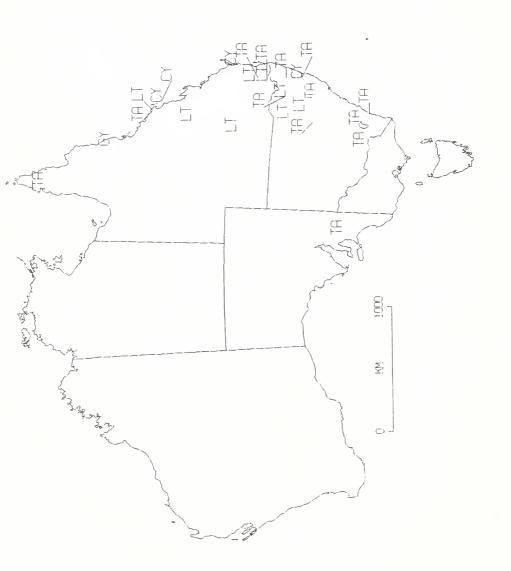
- At least a few accessions of each named species were included to provide a degree of replication at the species level;
- For each species, a geographic coverage of each Australian state in which it occurred was included, with as broad a scatter and range of habitats as possible;
- Known morphological, cytological and isozyme groups were represented from species in which intraspecific variation has been studied.
- 4. When choices were still to be made, preference was given to accessions that have been used in the past for research, or were authentic, firsthand collections.

Table 1 gives the number of accessions in the core collection for each *Glycine* species, according to the state of origin. This number is compared with the total number from that state in the whole collection. The figures show that in achieving the overall target of about 10% retention, the proportion of each category (species x state) has varied dramatically.

The full list of accessions in the core collection is given in Table 2. The table includes the G or Glycine number in the CSIRO Perennial Glycine collection, the PI or USDA Plant Introduction number if the accession has already been introduced into the United States, and the locality of origin. The distribution of the Australian accessions is shown in three figures. Figure 1 maps the distribution of the accessions of G. canescens (CA),







Core and total collections of perennial Glycine species, by state of origin (June 1986) Table 1.

Glycine species	Q1	q1d ^a	NSM	Μ	Vic	Tas	SA	WA	IN	Other
arenaria	1	_م			ı	ı	ı	3 4		1
argyrea	3	15	ı		ı	ı	1	ı	ı	ı
canescens		œ	9	65	1 1	ı	2 5	3	3 16	1
clandestina	3	20	11	158		1 1	2 3			ı
curvata	4	7	ı		ı	ı	ı	1	ı	ı
cyrtoloba	5	27	1	7	1	1	ı	ı	1	ı
falcata	5	23	1		ı	ı				1
latifolia	9	14	5	10	ı	ı	1	1	ı	ı
latrobeana		1	1		3 9	2 5	1 1	ı	ı	1
microphylla	2	14	2	85	1 2	1 1		ı	ı	
tabacina	5	42	∞	151			1 2		1	2 38
tomentella (2x)	4	52			ı	ı	ı	1 21		1 9
tomentella (4x)	2	139	3	40	ı	ı	ı	2 5	1 8	3 15

Abbreviations: QLD - Queensland, NSW = New South Wales, Vic = Victoria, Tas = Tasmania, SA = South Australia, WA = Western Australia, NT = Northern Territory, Other = South East Asia, Papua New Guinea, South Pacific Islands.

 $^{\mathsf{b}}_{\mathsf{A}}$ hyphen indicates that the species is not currently known to occur in that state.

Table 2. Perennial Glycine core collection (June 1986)

Acc. Number	Other numbers	Collection locality	State
		G. arenaria Tind. MS	
G1305		"Carlton Hill" Station, 50 km NW of Kununurra	WA
G1931		Kimberley Research Station Enclosure, 35 km N of Kununurra	WA
G1949		Weaber Range, 60 km N of Kununurra	WA
		G. argyrea Tind.	
G1420		Rainbow Beach, Cooloola National Park	Qld
G1622		Rainbow Beach, 3 km SE of	Qld
G1626		Cooloola National Park, 7 km N of Freshwater Camping Area	Qld
		G. canescens F. J. Herm.	
G1104	PI 440.934	Kings Park, Perth	WA
G1113	PI 440.936	Condoblin	NSW
G1120	PI 440.942	Lachlan Ranges, 16 km SW of Lake Cargelligo	NSW
G1232		Cobham Lake, 240 km N of Broken Hill	NSW
G1249	PI 483.191	Delalah/Thurloo Downs, 150 km E of Tibooburra	NSW
G1270		Coligram	Vic
G1301	PI 483.192	Alice Springs	NT
G1340		Murray Downs Station	NT
G1482		North Pool, Wiluna	WA
G1485		Peak Hill, 35 km S of	WA
G1500		Warialda	NSW
G1672		Stanley Chasm	NT
G1852	PI 440.931	Lake Torrens Basin, 40 km NW of Leigh Creek	SA

Table 2. Continued

Acc. number	Other numbers	Collection locality	State
G1853	PI 440.932	Goyders Lagoon, 65 km S of Birdsville	SA
G1966		Enngonia, 6 km N of	NSW
		G. clandestina Wendl.	
G1001		Black Mountain	ACT
G10 3 1		Tinderry Ranges, S of Queanbeyan	NSW
G1035		Lobs Hole Road, Cabramurra	NSW
G1145		Stannifer, 10 km N of Tingha	NSW
G11 7 5		Ellenborough Falls, 16 km E of	NSW
G1225		Kangaroo R. Bridge, 8 km E of Robertson	NSW
G1253	PI 483.194	Captains Flat	NSW
G1356		Kelso	Tas
G1558		Crackneck Reserve, Bateau Bay	NSW
G15 7 5		Casino Abattoirs	NSW
G1659		Stanthorpe, 12 km S of	Qld
G1664		Rainbow Beach, 3 km S of	Qld
G1826		Dingo Beach, 40 km N of Proserpine	Qld
G1873	PI 440.952	Brachina Gorge, 34 km SE of Parachilna	SA
G1874	PI 440.954	Moro Gorge, 20 km of Balcanoona	SA
G1899		Comerloy Road, 1 km N of	NSW
G1961		Hermidale, 1 km E of; 45 km W of Nyngan	NSW
		G. curvata Tind.	
G1396		Mareeba, 14 km SE of	Q1d
G1846		Weipa, 40 km E of	Q1d
G1848		Greenvale, 3 km W of	Qld
G1849		Homestead, 2 km W of	Qld

Table 2. Continued

Acc. number	Other numbers	Collection locality	State
		C. cyrtoloba Tind.	
G1184	PI 440.962	Brampton Island	Qld
G1185	PI 440.963	Brampton Island airstrip	Qld
G1236	PI 373.993	Grafton Agric. Res. Station	NSW
G1729		Rainbow Beach, 2.3 km S of	Qld
G1832		Wongabel, 10 km W of	Qld
		G. falcata Benth.	
G1153	PI 440.975	"Nunbank", 32 km NW of Taroom	Qld
G1155		"Lonogan", Tambo	Qld
G2082		Capella Creek, Capella	Qld
G2085		Comet River, 2 km W of Comet	Qld
G2086		Coruna Bore, 16 km N of Barcaldine	Qld
	G. lati	ifolia (Benth.) Newell & Hymowitz	
G1137	PI 440.978	Delungra	NSW
G1160		Warwick	Qld
G1179		Goombungee	Qld
G1213		Burren Junction, 10 km N of	NSW
G1343	PI 253.238	Capella	Qld
G1466		Mungallala, 8 km W of	Qld
G1827		Vine Creek, 18 km N of Proserpine	Qld
G1909		Mullaby, 15 km E and 49 km NE of Coonabarabran	NSW
	G .	latrobeana (Meissn.) Benth.	-
G1252	PI 440.977	Coleraine, Nareen Road	Víc
G1383		Mouchong Creek, 50 km S of Horsham	Vic

Table 2. Continued

Acc. number	Other numbers	Collection locality	State
G1387		Meruka Park, 25 km NE of Melbourne	Vic
G1389		Lagoon of Islands	Tas
G1900		Upper Ouse River, 6 km W of Miena	Tas
G1920		Mt. Crawford Forest Reserve	SA
	G.	microphylla (Benth.) Tind. MS	
G1143	PI 339.664	Grafton Agric. Res. Station	NSW
G1173		Ellensborough Falls	NSW
G1195	PI 446.939	Berry Mountain Road, Berry	NSW
G1272		Bunya Mountains, Dalby	Q1d
G1479		Cronulla, 25 km S of Sydney	NSW
G1555		Yen yean Reservoir, 30 km N of Melbourne	Vic
G1590		Woodenbong, 13 km W of	NSW
G1830		Tumoulin	Qld
G1901		Upper Ouse River, 6 km W of Miena	Tas
	. G. 1	tabacina (Labill.) Benth. (2x)	
G1138	PI 339.661	Cangai, 50 km W of Grafton	NSW
G1431	PI 373.986	Purgatory Creek, 35 km W of Grafton	NSW
G1538		Helidon, 5 km S of	Qld
G1545		Mt. Tamborine, 15 km W of Oxenford	Qld
		G. tabacina (4x)	
G1075		Mt. Painter, Cook	ACT
G1208		Broulee Beach	NSW
G1226	PI 446.974	Ryukyus, Myako Island	Japan
G1234		Tamworth	NSW
G1254		Morven, 2 km NE and 10 km E of Culcairn	NSW

Table 2. Continued

Acc.	Other numbers	Collection locality	State
G1258	PI 440.990	New Caledonia	NCL
G1262		Warwick	Q1d
G1314	PI 373.990	Delungra	NSW
G1503		Coonabarabran, $18\ \mathrm{km}\ \mathrm{S}$ of and $1\ \mathrm{km}$ along Mendooran Road	NSW
G1706		Mt. Crawford Forest Reserve	SA
G1828		Weipa, 40 km E of	Q1d
G1831		Vine Creek, 18 km N of Proserpine	Q1d
		G. tomentella Hayata (2x)	
G1300	PI 441.000	Mt. Garnett, 70 km SE of	Q1d
G1303		Stonewall Creek, 25 km NE of Ord Dam	WA
G1316	PI 373.987	Texas	Q1d
G1366	PI 446.995	Lae, 2 km from Erap Bridge	PNG
G1413		Mt. Garnett, 20 km SW of	Qld
G1780		Pine Tree Creek, 116 km N of Hughenden	Q1d
		G. tomentella (4x)	
G1133	PI 441.001	Brampton Island	Q1d
G1136	PI 441.006	Yimkin, Graman, 40 km N of Inverell	NSW
G1146	PI 441.003	Brampton Island Golf Course	Qld
G1304		Adelaide River	NT
G1347	PI 320.547	Kingmen	TWN
G1359	PI 446.988	Boroko, 3 km S of, Port Moresby	PNG
G1367	PI 446.996	Port Moresby	PNG
G1398		Emu Creek, Petford	Qld
G1487		Station Creek, 53 km SW of Narrabri	NSW

Table 2. Continued

Acc. number	Other numbers	Collection locality	State
G1744		False Pera Head, 60 km SW of Weipa	Qld
G1747		Along Creek, 20 km E of Weipa	Qld
G1934		Russ Creek, 5 km NE of Gibb Range	WA
G1969		Marthaguy Creek, 13 km W of Gilgandra	NSW
G2059		Grotto Creek, 32 km S of Wyndham	WA

G. arenaria (AR), G. curvata (CV), G. falcata (FA), G. argyrea (AG), G. microphylla (MP), and G. latrobeana (LB). Figure 2 maps the origin of the accessions of G. tomentella (TO) and G. clandestina (CL). Finally, Figure 3 maps those of G. tabacina (TA), G. cyrtoloba (CY), and G. latifolia (LT).

Evolution of the core collection

The core set of accessions is a dynamic rather than a static set. In the course of time, changes in content and size of the core are to be anticipated. The factors which will cause such changes are:

- Receipt of new accessions into the overall collection from distinctly new areas or of new taxa;
- 2. Replacement of accessions of questionable authenticity (if any) with new samples from a presumably comparable source;
- Revision of categories or affinities in the light of new data about accessions from evaluation, research, or "passport" information;
- 4. Review of breeders' needs.

However, alteration of the core ideally should be relatively infrequent. One aim of the core is to build up a body of information on a restricted yet comprehensive "reference" set of accessions, and too rapid a flux of accessions through the core would defeat this aim.

Conclusion

The setting up of the core collection for *Glycine* should facilitate the study and use of the perennial species in soybean improvement. Structured sampling of the wild gene pool will enable more specificity in requests for germplasm samples, efficient shipment of samples, and greater and quicker appreciation of the genetic diversity they represent.

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 - A. H. D. Brown
 - J. P. Grace
 - S. S. Speer

Brazil

1) Effects of seed color on seed deterioration.

It has been noted (Dassou and Kueneman, 1984) that soybean varieties with colored seed frequently show tolerance to seed deterioration. In Brazil, naturally occurring, colored-seeded mutants of four soybean lines have been found. The existence of these near-isolines provided an opportunity to assess the role of seed color on seed deterioration in similar genetic backgrounds.

In a preliminary study, the variety IAC-8 and its black-seeded isoline were sown on 27 December 1984 in single-row plots with four replicates at the experimental farm of Empresa Goiania de Agropecuaria, located 30 km south of Goiania, Goias, Brazil. On 18 April 1985, both lines were at physiological maturity and a sample of five to 10 plants per plot were removed and hung to dry in a ventilated shed. On the same date, 50 pods (yellow-pod stage) were subjected to incubator-weathering which mimics field weathering under humid conditions. Pods were stored at 30°C and 95% relative humidity for 7 days. Following incubator-weathering, 50 seeds from each plot were sown in an emergence test (Table 1). The seed deterioration following incubator-weathering was more drastic and the black-seeded isoline of IAC-8 had a significantly higher emergence score than that of IAC-8.

Table 1. Effect of incubator weathering on seedling emergence of yellowand black-seeded isolines of soybean variety IAC-8, 1985

Genotype	Physiological maturity	Incubator weathering
	% emerg	ence ———
IAC-8 IAC-8 black	96.0 92.5	23.0 37.5
LSD (0.05)*	14.4	14.4

^{*}Least significant difference.

In 1986, four pairs of isolines were evaluated for resistance to incubator-weathering and to modified accelerated aging which mimics seed deterioration in storage under ambient tropical conditions (Wien and Kueneman, 1981). Seed was stored for 6 weeks at $40\,^{\circ}\mathrm{C}$ and 75% relative humidity. Eight replicates were used to assess effects of incubator-weathering and four replicates were used for accelerated aging.

All colored-seeded isolines had significantly higher seedling emergence following incubator-weathering than their yellow-seeded versions.

Similarly, except for the pair GO 81-8181/GO 81-8181 black, colored-seeded lines were superior to their yellow-seeded versions following accelerated aging.

In the breeding program at the International Institute of Tropical Agriculture, many crosses have been made involving black-seeded cultivars from Indonesia with superior seed longevity and high-yielding varieties from the U.S. with poor seed longevity. Progenies were assessed for seed longevity in subsequent generations. Although some yellow-seeded breeding lines with excellent seed longevity have been obtained from these crosses, the majority of the breeding lines with good seed quality are colored-seeded.

The reasons why colored-seeded soybeans frequently have superior seed longevity need to be elucidated.

Table 2. Effect of seed color on seedling emergence following incubator weathering and accelerated aging, 1986

Genotype	Physiological maturity	Incubator weathering	Accelerated aging
		— % emergence —	
EMGOPA 301-1	95	17	9
EMGOPA 301-Brown	89	35	76
Doko	98	56	38
Ooko-black	93	75	76
AC-8	94	9	44
AC-8-black	85	19	83
GO 81-8181	77	12	38
GO 81-8181-black	88	33	36
LSD (0.05)*	6	6	12

^{*}Least significant difference.

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 Varietal differences in soybeans for resistance to physical damage of seed.

Introduction: Production of quality soybean seed is frequently problematic in the tropics (Jalani et al., 1982; Khaleque, 1982; Mercer-Quarshie and Nsowah, 1975; Ndimande et al., 1981; Paschal and Ellis, 1978). In general, loss of seed vigor is associated with seed deterioration in the field prior to harvest (Ellis and Sinclair, 1976; Green and Pinnel, 1968; Ndimande et al., 1981; Paschal and Ellis, 1978; Potts et al., 1978) and with deterioration in storage (Kueneman, 1982; Minor and Paschal, 1982; Ndimande et al., 1981; Tongdee, 1982; Wien and Kueneman, 1981); seed deterioration is accelerated by hot, humid environments. While there is considerable scope for development of genotypes less prone to deterioration (Dassou and Kueneman, 1984; Kueneman, 1982, 1983; Paschal and Ellis, 1978; Wien and Kueneman, 1981), it is generally recommended that soybeans grown for seed should be sown such that they mature under dry environmental conditions. Often in the tropics, seed production must take place in the dry off-season under irrigation, which increases costs of production.

In Brazil, many seed growers have been frustrated by rejections of seed lots due to low germinability even though the seed was produced under favorable environmental conditions. Analyses of seed lots have shown that loss of vigor is often due to physical damage of seed. Physical damage to seed occurs from a series of mechanical operations beginning at harvest and occurring during seed handling until seed is sown in the subsequent season (Nave, 1980; Huynh et al., 1980); the greatest damage generally occurs at threshing. Combines with a relatively gentle threshing action have been developed (Nave, 1980) but are costly and not readily available in many developing countries. Sediyama et al. (1972) noted that variety IAC-2 was more prone to mechanical damage than 'Mineira' and 'Vicoja'. The authors know of no other studies comparing soybean varieties for resistance to physical damage.

In Brazil, seed scientists, producers, and farmers have had the impression that some varieties are more prone than others to physical damage. This study was conducted to determine if there are differences among varieties grown in central Brazil for resistance to physical damage.

Materials and methods: Eighteen soybean varieties adapted to central Brazil were sown on six dates: October 20 and 30; November 10 and 20; December 10 and 30, 1984, and again in 1985. The trial was conducted at the experimental station of Empresa Goiania de Pesquisa Agropecuaria located 30 km south of Goiania, Goias, Brazil. Plots were four rows of 6 m length. Plots

were oversown and thinned to approximately 20 seedlings per m of row at 14 d after sowing. The design was a completely randomized block with four replicates, each block with 108 experimental units (18 varieties x six planting dates).

On 15 April, all varieties within a block reached physiological maturity (yellow pod stage). Late maturing varieties were at physiological maturity in earlier planting dates; earlier maturing varieties were at physiological maturity in later plantings. For each variety in each of four blocks, a sample of 200 plants was collected 21 days after physiological maturity and stored in a covered, ventilated shed until seed of all varieties equilibrated at approximately 12% moisture content. Five plants were hand-threshed and the seed sown in emergence tests to ensure that all varieties were of high quality prior to being subjected to physical stress. The remaining plants were passed through a spike-toothed vogel-type plot thresher and percentage of broken seed was determined.

Results and discussion: Percentage seedling emergence of hand-threshed seed was 90% and above for all varieties in 1984/85 (Table 1) indicating that seed entering the physical stress was of high quality. The difference among varieties for percentage of broken seed was highly significant (Table 1). 'Cristalina', 'Doko', 'EMGOPA 301', 'EMGOPA 302', and 'Bossier' had low percentages of broken seed in both years and appear to be relatively resistant to physical damage. IAC-8, IAC-2, IAC-6, UFV-1, and 'Sucupira' appear to be prone to seed damage. Varieties IAC-7, EMGOPA 303, 'Santa Rosa', 'Paranagoiana', 'Tropical', and 'Savana' appear to have intermediate susceptibility. Results were not consistent across years for varieties 'Numbaira' and 'Parana'.

Seed weights among varieties ranged from 12 to 19 g per 100 seeds and there was no clear relationship between seed size and percentage of broken seed (Table 1).

In this study, IAC-2 was one of the most susceptible varieties to physical damage. This is consistent with previous reports (Sediyama et al., 1972). With a ninefold difference between the most resistant and most susceptible varieties in this study, there appears to be great scope for selection of varieties less prone to damage. In ecologies where seed is produced under dry conditions and physical damage of seed is common, it is recommended that breeders evaluate varieties and breeding lines for resistance to physical stress and that lines with high susceptibility be discarded unless combines with gentle threshing action are available.

Table 1. Seed weight, percentage emergence of hand-threshed seed and percentage broken seed following mechanical threshing for 18 soybean lines adapted to central Brazil

	Broker	n seed	Seed weight	Emergence
Genotype	% 1985	% 1986	(g/100 seed)	(%)
IAC-8	31.0	20.5	18	96
IAC-2	29.5	25.0	14	91
Numbaira	29.2	9.3	14	96
IAC-6	26.9	17.8	12	97
UFV-1	26.3	14.0	17	99
IAC-7	19.9	17.2	13	96
Sucupira	19.5	33.6	16	93
EMGOPA 303	16.8	13.7	13	95
Santa Rosa	13.6	12.9	15	95
EMGOPA 301	12.0	6.4	17	97
Paranagoiana	11.6	11.6	15	93
Tropical	9.6	18.8	15	97
Savana	7.1	14.6	16	98
Doko	6.8	8.8	15	98
Parana	6.3	22.4	13	96
EMGOPA 302	5.2	12.8	14	96
Cristalina	4.0	7.8	16	95
Bossier	3.5	12.2	19	90
LSD (0.05)*	6.6	12.2	-	7

^{*}Least significant difference.

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Linkage tests.

Results reported by Buzzell (1974) were not conclusive as to whether or not E3 (daylength response) and Ep (seedcoat peroxidase) are independent or loosely linked. Palmer et al. (1984) has shown that Ep is linked with Fr1 in linkage group 12.

Using F_2 plants of 'Minsoy' x 'Hark' previously reported (Palmer et al., 1984) for seedcoat peroxidase and root fluorescence, we tested for linkage of E3 with Ep and Fr1 by testing F_3 material for response under a daylength extended to 20 h with cool white fluorescent light. In addition, the material was evaluated for resistant (Rmd)/susceptible (rmd) reaction to powdery mildew.

'Blackhawk' carries the e3 and Rmd alleles and Hark the E3 and rmd alleles. Both Blackhawk and Minsoy are early flowering and resistant to mildew. Therefore, an allelism test was made by crossing Blackhawk x Minsoy. For daylength response, there were 122 flowering plants and no nonflowering plants; for powdery mildew reaction there were 92 resistant plants and no susceptible plants. Minsoy appears to carry the same e3 and Rmd alleles as Blackhawk.

Results (Table 1) indicate that the $\it E3$ and $\it Rmd$ are not linked with $\it Ep$ and $\it Fr1$ in linkage group 12. $\it E3$ and $\it Rmd$ may be loosely linked, however; further evaluations will be done to test whether or not these genes are linked or are independent.

						+
Table	1.	Results	o f	F2	linkage	tests

		Numb	er of F	2 plant	s			
Genes	а	ь	С	d	Sum	%R	SE	Phase
М	insoy (ep	fr1	e3 Rmđ)	x Hark	(Ep Fr1	E3 rmd)		
E3 e3 Ep ep	207	50	67	13	337	53.0	4.2	С
E3 e3 Fr1 fr1	205	51	59	21	336	45.0	3.9	С
E3 e3 Rmd rmd	177	53	50	7	287	39.3	4.9	R
Ep ep Rmd rmd	184	50	45	8	287	I		R
Fr1 fr1 Rmd rmd	183	42	46	16	287	I		R
	Haros	oy (£	g3 Pc) :	x L63-10)97 (Fg3	pc)		
Fg3 fg3 Pc pc	36	13	15	4	68	45.8	9.5	R

Product method, Immer and Henderson (1943).

 $^{^{++}}$ C = Coupling; R = Repulsion.

Buzzell and Palmer (1985) reported that Fg3 and pc might be closely linked. The 'Harosoy' isoline L63-1097 was crossed to Harosoy; the presence of Fg3/fg3 was determined by thin layer chromatography by using leaf samples of F_2 plants and the presence of Pc/pc (pubescence type) was determined by using F_2 plants and confirmed by F_3 tests grown in the greenhouse. Results (Table 1) indicate that the presence of Fg3 in L63-1097 after five backcrosses for pc is a chance occurrence and not the result of a close linkage.

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1) Harosoy Rps isolines.

The development of Rps isolines of 'Harosoy' has been reported (Buzzell et al., 1984). The Rps1-a and Rps5 isolines were available as 'Harosoy 63' and L62-904, respectively. Isolines have been completed for Rps1-b, Rps1-k, Rps3, Rps4, and Rps6. Rps1-c is at BC $_6$ and Rps3-b is at BC $_1$.

The completed isolines have been coded by a two-digit number (Table 1); the first digit signifies the locus and the second digit designates the allele with 1 representing rps. Harosoy is HARO (1-6)1, i.e., recessive at locus 1 to 6 and Harosoy 63 is HARO 12, i.e., Rps1-a. The gene Rps? (Rennie and Buzzell, 1986) which confers resistance to races 12, 16, 18 and 19 is indicated by XX, i.e., uncharacterized for locus and allele.

The Rps isolines were evaluated for reaction to available races (20 and 23 not available) and the results (Table 1) compared with published (Buzzell et al., 1984; Keeling, 1982, 1984; Laviolette and Athow, 1983; Layton et al., 1986; Moots et al., 1983; Ploper et al., 1985) and unpublished reactions. Since Harosoy carries Rps?, reactions for races 12, 16, 18 and 19 are not included in the table. Our susceptible reaction for race 14 and Rps1-a agrees with that of Moots et al. (1983) and Athow (personal communication, 1984) but not with the resistant reaction reported by Keeling (1982) and Laviolette and Athow (1983). Athow (personal communication, 1984) pointed out that with a susceptible reaction for race 14 and Rps1-a, races 4 and 14 are the same. These two races have the same reactions in Table 1.

The rps? allele is being backcrossed into Harosoy so that single gene Rps isolines can be developed. However, this may have already been accomplished with Rps1-b. Five BC_6F_2 plants (from the same F_1 plant) selected as being Rps1-b Rps1-b are resistant to race 18 and susceptible to races 12, 16 and 19. Rps1-b confers resistance to race 18 and susceptibility to races 12, 16 and 19; thus, it appears that HARO 13 is rps? rps?. This seems an unusual chance occurrence; however, Rps? should be at a different locus than Rps1 (Rennie and Buzzell, 1986). The two loci may be linked; however, we did obtain an Rps1-b Rps? BC_6F_2 line from a different F_1 plant than the one which resulted in the Rps1-b rps? combination.

The backcrossing of an Rps gene from PI 103091 into Harosoy has been completed but the locus has not been characterized. The line gave a resistant reaction to races 6 and 7, in contrast to PI 103091, in which a susceptible reaction for race 6 and a resistant reaction for race 7 were reported by Laviolette and Athow (1983). The cultivar 'UCO 112' gives a susceptible reaction to race 6 and a resistant reaction to race 7; thus, it could be used as a differential to distinguish these two races.

Seed of the Harosoy Rps isolines will be available from the Harrow Research Station after seed increase in 1987.

Table 1. Reaction of Rps lines of soybean to races of Phytophthora megasperma f. sp. glycinea

Line(s) tested	Gene	Source of gene	No.* 1		2	m	4	5	. 9	_	∞	9 1	Race 0 11	- Race	3 17		5 1		21	22	24	25
HARO (1-6)1XX	Rps?	Harosoy	'	t _o	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	Rps1-a	Blackhawk	∞	R	R	S	S	S	S	S	S	S	R	R	W W	S	R	R	S	S	R	S
	Rps1-b	PI 84637	7	R	S	×	R	×	×	R	×	R	S	S	~	×	R	S	×	R	S	S
	Rps1-c	PI 54615-1	2	ĸ	ĸ	Ж	S	S	×	R	R	R	×	R	M S	S	×	×	×	S	×	S
	Rps1-k	Kingwa	7	ĸ	Ж	ĸ	ĸ	ĸ	ĸ	R	В	R	×	R	R	24	×	×	×	×	×	S
	Rps3	PI 171442	7	K	R	Ж	ĸ	K	S	S	R	R	S	R	R	×	S	S	S	S	S	\simeq
PRX146-36/47**	Rps3-b	PI 172901	-	ĸ	R	ĸ	×	×	×	R	×	R	×	R	R	×	×	S	×	R	×	K
	Rps4	PI 86050	7	R	×	ĸ	R	S	S	S	S	S	R	S	R I	×	~	S	×	S	S	R
	Rps5	PI 91160	∞	K	Ж	ĸ	R	×	S	S	В	×	S	~	×	×	S	S	S	S	S	R
	Rps6	Altona	7	ĸ	ĸ	ĸ	R	S	S	S	S	S	R	S		×	×	S	R	S	S	ĸ

*No. of crosses with Harosoy.

**Lines from K. L. Athow.

+S = Susceptible; R = Resistant.

*An interim line.

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1) On the response of the Rps1-b allele to race 17 of Phytophthora megasperma f. sp. glycinea.

The first report of the interaction between race 17 of Phytophthora megasperma f. sp. glycinea (Pmg) and the Rps1-b allele was made by Keeling (1982) in his initial report on this race. Keeling (1982) reported that 'Sanga' (Rps1-b) gave a resistant response to race 17, but that PI 171442 (Rps3) and 'Tracy', which possesses the Rps1-b and Rps3 alleles (Athow et al., 1979), gave a susceptible response. The relative responses of Tracy and Sanga were unexpected, since, in all cases studied to date, an Rps allele for resistance is epistatic to Rps alleles for susceptibility at any loci (e.g., Athow et al., 1980; Layton et al., 1984; Ploper et al., 1985; Athow et al., 1986).

Laviolette and Athow (1983) and Athow (1985) appeared to confirm the pattern reported by Keeling (1982), but it is not clear whether this was the result of independent experimentation, or merely a repetition of the table from Keeling. Buzzell et al. (1984) advised other researchers that their preliminary information did not agree with the published response for Rpsl-b and race 17.

Following the World Soybean Research Conference III in 1984, Dr. Athow sent a copy of the race-response pattern of the various Rps alleles to most of the known races of Pmg to workers dealing with this disease. In this review, he categorized the response of Sanga (Rps1-b) to race 17 as being susceptible (S). The current work was designed to fully clarify this interaction.

In the current work, Sanga (Rps1-b), 0X681 (Rps1-b), Harosoy-Ne_B [a line derived from 'Nezumisaya' X 'Harosoy'] (Rps1-b), Tracy (Rps1-b, Rps3) and PI 171442 (Rps3) were compared for response to race 17. Table 1 shows that each line possessing Rps1-b or Rps3 or both displays a susceptible reaction to race 17. The identity of race 17 is confirmed by its reaction to the other lines in the differential host series.

It appears that the actual response of the *Rps1-b* allele to Pmg race 17 is an S (susceptible) reaction. The reasons for the results that occurred in the original work of Keeling (1982) are not known. It is important to clarify the actual situation regarding this race response in order that breeders do not count on a source of resistance that isn't real (i.e., *Rps1-b* as a source of resistance to race 17).

Table 1. Response of soybean lines to inoculation with Phytophthora megasperma f. sp. glycinea race 17

Line	Allele	Observed ^a	Reaction
Harosoy	Rps?	16/16	S
Harosoy 63	Rps1-a + Rps?	2/17	R
Sanga	Rps1-b	41/41	S
Mack	Rps1-c	0/31	R
PI 171442	Rps3	29/29	S
Altona	Rps6	17/18	S
PI 103091	???	37/37	R
Tracy	Rps1-b + Rps3	20/20	S
OX681	Rps1-b	41/42 (54/65	s) ^b s
Harosoy-Ne _B	Rps1-b + Rps?	99/99	S

aObserved number of susceptible plants/total number inoculated.

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bData from Buzzell et al., 1984.

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1) Studies on genetic variation of soybean agronomic characters induced by seed irradiation.

Since the 1950s, there have been a lot of geneticists who studied the genetic variation of the populations derived from irradiated seeds in the world, but only a few who studied the principle of selection for these populations. Some breeders have not only developed new varieties by using irradiation, but also have studied the genetic variation of irradiated populations. However, the populations that they used had been often selected; therefore, it was hard to avoid that the results had been influenced by directional selection effect. The purpose of this work was to study the variation, heritability, and genetic advance of major agronomic characters in M2 and M3 by using random sampling populations derived from seed irradiation in order to know the genetic variation regularity of major agronomic characters of soybeans and try to find out the best suitable generation and focal point of the characters for selection in irradiated populations.

Materials and methods: The experiment was laid out consecutively for three years, 1979-1981, in nested design with four replications. Dry seeds of three soybean varieties were irradiated by 60Co-r ray with a dosage of 16KR. The varieties irradiated were 'Fengshou 10', 'Dongnong 74-403', and 'Heinong 26', and nonirradiated seeds of the corresponding varieties were used as control. Three types of plants, semisterile (MS), fertile (MF) and control (CK), were harvested randomly in M1 to derive M2 and M3 populations. Ten plots were harvested for each type in 1979 (M1); 30 lines were planted in 1980 (M2); then, half of those lines was planted to get M3 family populations. There were five lines for each family, five families for each type population, and three type populations (MF, MS, and CK) in 1981 (M3).

The genotypic variance was estimated by using variance of CK as environmental variance and the variance of MF and MS populations as phenotypic variances. On this basis, the broad sense heritability was estimated. The genetic advances expected through selection and relative genetic advance were estimated as described by Robinson et al. (1961) and Johnson et al. (1963), respectively.

Results and analyses: 1. The frequency distribution curve of every agronomic character in irradiation populations was more expansive than those of CK. It showed that after irradiation new variances of major agronomic characters occurred in the irradiated populations. The characters that show a higher level of genetic variance were number of branches, seeds per plant, pods per plant, and yield of plant, GVC=20-30%. Seed weight and ratio between seed and stem weight were middle in the level of genetic variance, GVC=10-20%. The characters with the lowest level of genetic variance were plant height, number of nodes of main stem, and growing date, GVC<10%. The result of M3 was similar to those of M2, but the frequency of variance was somewhat lower.

- 2. In M2, the characters with high levels of heritability were growing date and 100-seed weight. The value of their heritabilities was about 80%. The characters with middle level of heritability, about 60%, were pods per plant and nodes of main stem. The characters with lower level of heritability, less than 40%, were plant height, branch, seed per plant, ratio between seed and stem weight, and yield per plant. In M3, the heritability of ratio between seed and stem weight was second when it was estimated by using lines as the unit, and that of plant height was second when estimated heritabilities by using the correlation between M2 and M3.
- 3. Some selection advance for every character could be obtained when the selection had been carried out as a selection ratio with 5% in irradiated populations; that was more than 30% for branch, seeds per plant, pods per plant, yield per plant, and 100-seed weight; that was around 20% for ratio between seed and stem weight, plant height and nodes of main stem. The relative genetic advance of growing date was the lowest. It was less than 10% (see Figure 1).
- 4. In M2, it was effect that characters of growing date and seed weight, which heritabilities were higher, had been selected. The actual gain was about 25% of that of expected value, and the selected effect to get early maturing and big seeds was higher than that to get late maturing and small seeds. But, it was not effect that selection had been carried out for yield of plant with lower level of heritability. The actual gain was only one-thirtieth of expected value (see Table 1).
- 5. There was no immediate relationship between Ml and M2 in fertility and major agronomic characters. When the normal plants of M2, which derived from semisterile plants, were planted, all of the plants of M3 were normal in fertility. Performances of major agronomic characters showed similar as that of the progenies which came from normal plants of Ml.
- <u>Discussion</u>: 1. Under irradiation treatments, all nine agronomic characters performed definite genetic variation. The estimates of the phenotypic variance of irradiated plants averaged two times as large as those of controls. The variations were heritable. Genetic advance expected through selection can be obtained when selection was exerted upon the irradiated populations. The magnitude of estimates of heritability of all the characters in the M3 became smaller in comparison with those of M2, and such characters tended to become stable in M3.
- 2. M2 is the crucial generation for selection, because irradiated populations varied more extensively and thus had greater potential for selection. Some characters had larger heritability value in M2, and their correlation values between M2 and M3 were also significant.
- 3. Selection may be taken for growing date, 100-seed weight, and plant height in M2, because they had larger heritability in that generation, and correlation between M2 and M3 was significant. The performance of these characters in M2 may be represented in M3. In M3 reliable selection can also be taken for ratio between seed and stem weight. Selection for other characters seems not to be justified before M3.

4. The value of all the genetic parameters of the progenies of MS were bigger than those of MF. More extensive variability, greater heritability and greater genetic advance expected through selection were obtained from progenies of MS. Most plants of the progenies of MS in M1 can recover to normal fertility in the future generations. Plants of MS in M1 are more valuable for mutation breeding.

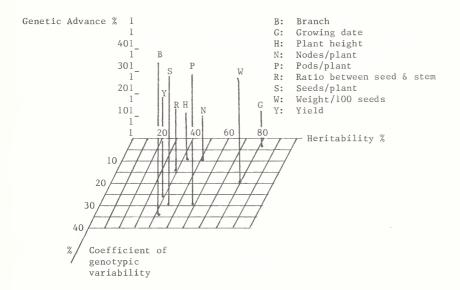


Figure 1. Relative genetic advance of irradiated population

Table 1. Effect of selection on several main agronomic characters in M2

Character	Variety	Heritab. in M2	Direction selected	Gain through selection	Actual gain in M2
Growing date	Fengshou 10	84.44	Early maturity	2.1	3.0
100-seed weight	Fengshou 10	81.96	Big seed	10.4	2.6
Plant yield	Dongnong	24.10	High yield	48.1	0.4

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1) Alkali-tolerant soybean mutant obtained by means of irradiation.

Some soybean mutants have been selected in our soybean mutation breeding program in recent years. They differed from parents (untreated) characterized by early maturity, high yield, good seed quality, and resistance to environmental stress. In the course of the selection, it was found that irradiation had obvious effects of induced variation upon the change of days to maturity and the improvement of the seed quality as well as its morphology. It also produced synthetic effects for increasing lodging tolerance and seed yield. There also were variations in pubescence growth, pod-bearing, foliar size and shape, etc. All of the mutants may be useful germplasm sources for soybean development.

This report wants to show a soybean alkali-tolerant mutant, 'Longfu 73-8955', which developed from 'Fengshan No. 1' irradiated with gamma rays. The original variety, Fengshan No. 1, had good yield components (larger branches, more pods/plant, larger seed size), resistance to lodging, but did not ripen before frost in Harbin. In order to shorten the days to maturity and to maintain other good agronomic characteristics, air-dried seeds of Fengshan No. 1 were irradiated with 9k rad of Co60 gamma rays in 1970. In $\rm M_1$ generation, surviving plants were harvested individually in three kinds: fertile, sterile, and semi-sterile (<50% seed set) plants. The $\rm M_1$ plants all were planted for plant-to-row progenies for $\rm M_2$ population with the above plant types and Fengshan No. 1 as control in the same conditions.

In M $_3$ population, some individual plants were selected from treatment and control (adapted cultivar) in special goal. The family of Longfu 73-8955 was obtained in M $_4$ progeny of the fertile plant in M $_1$. In 1974, it was tested on salt-alkali cropland of pH = 7.2-8.2, Zhaodong county, the southern Heilongjiang province. The result was 1800-1920 kg/ha yield, and 3-5 days earlier maturing than Fengshan No. 1, also salt-alkali tolerance was better, and other agronomic characteristics were similar to original variety.

The test of alkali-tolerance for the mutant and its original variety was conducted with pots under natural conditions in 1984. The soil used was transported from Zhaodong county. Four treatments of total alkalinity were designed: 0.203 (ck), 0.565, 0.610, 0.897 me/100 g soil, randomized complete block with four replications. Data were collected for emergence rate, survival plant rate, maturity, plant height, total seed wt/plant, dry wt/stem and wt/100 seeds. The study showed emergence rate, days to maturity, survival plant rate and wt/100 seeds appeared to have no significant difference; plant height, total seed wt/plant, wt/stem, ratio of the latter two traits tended to reduce as total soil alkalinity increased.

1. There was no obvious difference for total seed wt/plant, of two materials in alkalinity of 0.203 me/100 g. Under the alkalinity of 0.610, it was not significantly reduced for the mutant, and Fengshan No. 1 decreased by 27.7% (p<0.05). In 0.897, the difference between the mutant and the original variety was highly significant (p<0.01). The mutant was 13.4% higher than Fengshan No. 1 for total seed wt/plant.

- 2. Plant height of the mutant lowered as the alkalinity increased (r = 0.7807) by 18.5% compared with control 1.5% (r = 0.0631) in 0.897 me/100 g soil.
- 3. The ratio of total seed wt/plant and wt/stem at maturity for the mutant rose (r = 0.4722) although its height lowered. Fengshan No. 1 in contrast to the mutant was significantly reduced (r = 0.8641) as alkalinity of soil increased (Table 1).

Table 1. Agronomic characteristics of the mutant compared with Fengshan No. 1 in different alkalinities (HCO_2) (Harbin, 1984)

Material	Total alkalinity (me/100 g)	Plant height (cm)	Total seed wt/plant (g)	Dry weight per plant (g)	Ratio*
Longfu 73-8955	0.897 0.610 0.565 0.203	54.67 53.58 68.96 67.08	16.62 19.53 20.26 19.98	6.11 7.27 8.24 7.68	2.72 2.69 2.46 2.59
	r	-0.7807	-0.7827	-0.6939	0.4722
Fengshan No. 1	0.897 0.610 0.565 0.203	58.58 62.33 67.60 59.50	14.60 17.65 20.95 20.20	5.15 5.67 7.35 5.93	2.83 3.11 2.85 3.41
	r	-0.063	-0.8120	-0.3391	-0.8641

Data were collected at maturity.

Soybean is sensitive to salt-alkali concentration of soil, generally, pH = 6.5-7.5 is available for it. It is growing well under the salt concentration <0.15%, total alkalinity ($CO_3 + HCO_3$) < 0.6 me/100 g soil. The total salt concentration of soil used was 0.018-0.0083% in this test so total alkalinity was important factor for crop growth.

The results indicated that inducing mutation can be a method for developing soybean variety for salt-alkali soil.

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^{*}Ratio of total seed wt/plant and wt/stem.

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1) Inheritance of bloom on seed coat in soybean.

Woodworth (1932, 1933) reported that three dominant genes (B_1 B_2 B_3) were necessary for the development of bloom on seed coat. Tang and Tai (1962) and Tang and Li (1964) pointed out that, in interspecific cross, *Glycine max* x *G. formosana*, complementary genes for bloom on seed coat existed, and the genetic constitutions for *G. max* were B_1 B_1 b_2 b_3 b_3 , those for *G. formosana* were B_1 B_1 B_2 B_2 B_3 B_3 . The purpose of this experiment was to give a further study on this character with more and new parental materials.

Materials and methods: Four parents (three species) were used in this experiment. They are listed in Table 1.

Table 1. Parents

Parents	Species	Bloom on seed coat	Growth habit	100-seed weight
Dongnong 4	G. max	No bloom	Erect	19.76
Dongnong 33	G. max	No bloom	Erect	25.97
Ji-50192	G. soja	Bloom	Vining	1.78
Long 79-4004	G. gracilis	No bloom	Vining	4.93

Four crosses were made at Harbin in 1985. After harvest, the seeds were classified as "No bloom" or "Bloom."

Results and discussion: The results are shown in Table 2 and Table 3.

In the first two crosses, it reveals that a single gene controlling bloom on seed coat is apparent. In CIII and CIV, two pairs of complementary genes are suggested to control the inheritance of bloom on seed coat. The observed number has a good fitness to the expected number.

According to previous studies, the genetic constitutions for bloom on seed coat of the parents used here are proposed as follows:

Table 2. Characteristics of bloom on seed coat of parents and F_1 generation

		- Characters ——	
Crosses	Female parent	Male parent	F ₁
Dongnong 4 x Ji-50192 (CI)	Nª	ва	В
Dongnong 33 x Ji-50192 (CII)	N	В	В
Dongnong 4 x Long 79-4004 (CIII)	N	N	В
Dongnong 33 x Long 79-4004 (CIV)	N	N	В

 $^{^{\}rm a}{\rm N},$ B represent "no bloom" and "bloom" on seed coat, respectively.

Table 3. The inheritance of bloom on seed coat

				F ₂	
Cross	es	Expected	Observed	χ2	P
CI	B ^a N ^a	78 (3) 26 (1)	76 28	0.1154	0.50-0.75
CII	B N	78.75 (3) 26.25 (1)	85 20	1.6794	0.10-0.25
CIII	B N	57.95 (9) 45.06 (7)	56 47	0.0818	0.75-0.90
CIV	B N	57.375 (9) 44.625 (7)	57 45 0.0006		> 0.90
		***	Test	cross ———	
CI	B N	16 (1) 16 (1)	17 15	0.0313	0.75-0.90
CII	B N	21.5 (1) 21.5 (1)	19 24	0.3721	0.50-0.75

^aN, B represent "no bloom" and "bloom" on seed coat, respectively.

If G. formosana is G. soja, our hypothesis of genetic constitutions for G. soja is different from that of Tang and Tai (1962) and Tang and Li (1964), and G. gracilis had not been included in their studies. In the previous studies, it seems that they all supposed that one species had the same genetic constitution for bloom on seed coat, but our results indicated that it was possible to have different genetic constitutions for bloom on seed coat in the same species. In the studies of bloom on soybean seed coat, this is the first time to report that bloom seed coat has been obtained from crossing between two parents of smooth seed coat.

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2) Inheritance of some agronomic characters of interspecific crosses in soybeans.

An attempt was made to investigate the inheritance, variation, and segregation of some agronomic characters of interspecific crosses in soybean. Effect of backcrossing with cultivated soybeans as recurrent parents was analyzed in order to find out reasonable and effective methods for utilizing wild and semi-wild soybean germplasms.

The parents used in this study were Glycine max (L.) Merr. (Dongnong-33, Dongnong 4), G. gracilis Skvortz. (Long 79-4004, Long 79-3407-1) and G. soja Sieb. and Zucc. (Ji-50192). Six combinations were made: Dongnong 4 x Ji-50192 (CI); Dongnong 33 x Ji-50192 (CII); Dongnong 4 x Long 79-3407-1 (CIII); Dongnong 33 x Long 79-3407-1 (CIV); Dongnong 4 x Long 79-4004 (CV); and Dongnong 33 x Long 79-4004 (CVI).

Parents and F_1 , F_2 , F_3 , B_W , B_C , BF_2 generations of the six crosses were planted in randomized block design at Harbin in 1985. Parameters of segregation, mode of inheritance, gene effects, phenotypic and genotypic correlations, heritability, genetic advances and selection indices of some agronomic characters were estimated. A part of the statistical parameters was shown in Tables 1-4.

The conclusions drawn from the present study are as follows:

- 1. \mathbf{F}_1 generation of interspecific crosses is not completely fertile.
- 2. Epistatic gene effect is a commonly existing and important genetic component of the characters studied here.
- There existed a wide range of variation and a high value of genetic advance of the characters studied. Transgressive segregation was observed

- in F_2 and F_3 for all the characters studied here except 100-seed weight and diameter of main stem. So, potential of selection for new types is high in soybean interspecific crosses.
- 4. High heritability has been found on days from planting to flowering, flowering to maturity, planting to maturity, and protein content, weight of 100 seeds, seed shape indices, plant height. Therefore, selection is effective on such characters in early generation.
- 5. In interspecific crosses, protein content of *G. soja* and *G. gracilis* is dominant or partially dominant over that of *G. max*. Additive and dominant gene effects are of the same importance to protein content, and epistatic gene effect is important, too. In certain crosses, selection for protein content based on selection indices is more effective on protein content itself.
- 6. Vining growth habit is a quantitative character controlled by a few pairs of genes.
- 7. The key point for utilizing wild and semi-wild soybean germplasms lies on choosing appropriate parental materials for crossing.
- 8. Backcrossing using G. max as recurrent parent is an effective method to utilize wild and semi-wild soybean germplasms. However, when the main purpose is to transfer high protein content to the recurrent parent, G. max, a limited number of cycles of backcrossing is suggested. If parents used are appropriate, it is possible to gain desirable lines through one or two cycles of backcrosses.
- 9. From a long-term point of view, it seems that the high protein content and some other desirable characters of wild soybeans (G. soja) are very valuable. However, using semi-wild soybeans (G. gracilis) or exotic cultivated soybeans (G. max) possessing high protein content as parent-al materials to develop new lines or cultivars seems to be more effective and more rapid.

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Table 1. Continued

Characters		* M	ъ °С	* #	F ₁	F ₂	F3	B *	B *	BF ₂
Days flowering to maturity	CV	55.0	86.5	70.8	79.1	70.8	70.6 11.35	61.4 10.69	76.3 7.38	78.2 8.08
Days planting to maturity	CV	115.0	135.1	125.1	133.6	122.1	122.0	123.4	125.3	125.4

 $^{\star}P_{W}$, $^{R}P_{c}$, $^{R}P_{m}$, $^{B}P_{w}$, $^{B}P_{c}$, and $^{B}F_{2}$ represent Ji-50192, Dongnong 33, value of middle parent, Ji-50192 x (Dongnong 33 x Ji-50192)) $^{F}P_{2}$, $^{B}P_{2}$ respectively.

**M and CV represent mean and coefficient of variation, respectively.

Table 1. Performances of progenies of cross Dongnong 33 $\times\ \mathrm{Ji-50192}$

Characters		* ¼	P *	P * m	H L	F.2	E E	B *	B *	BF ₂
Percent protein	M** CV**	45.85	44.57	45.21	45.96	46.38	45.50	46.90	44.61	43.44
Seed wt. per plant	M(g) CV	9.48	47.12	28.30	61.74 41.06	39.19 58.78	34.15 66.15	29.97 61.30	52.51 36.51	38.09
No. seed per plant	M	576.0	180.9	378.5	737.5 35.87	608.2 60.80	562.1 47.06	869.5	352.1 41.65	286.9 37.94
100-seed weight	M(g) CV	1,78	25.97	13.88	7.97	6.61 26.09	6.33	3.74	12.99	13.36
Seed shape index	M CV	2.06	1.21	1.64	1.77	1.75	1.67	1.95	1.48	1.43
Ratio of seeds to stems	M CV	0.6206	0.5886	0.6046	0.5698	0.6443	0.6328 14.31	0.6394	0.6181	0.6704
Plant height	M(cm) CV	109.5	8.06	100.2	187.8 12.11	137.2 20.40	137.7	134.3 16.92	124.7 20.51	112.3
No. branches	M CV	32.5	5.6	19.1	37.1 28.64	25.6	21.8 44.84	39.3 30.03	17.0	11.5 28.98
Diameter of main stem	M (mm)	2.25	11.40	6.83	5.89	5.49 18.96	5.82	3.77 17.87	8.44	8.47 18.55
Days planting to flowering	M CV	0.09	48.6	54.3	54.5	51.5	51.5	58.0 11.47	49.2 16.47	47.3

Table 2. Performances of progenies of cross Dongnong 33 x Long 79-4004

Characters		P_w*	P_c*	P _m *	$^{\mathrm{F}}_{1}$	F ₂	F ₃
Percent protein	CV**	42.21	44.57	43.39	42.59 1.87	42.08 5.36	
Seed weight per plant	M(g) CV	17.53	47.12	32.32	66.89 35.75	40.87 45.49	39.23 36.94
No. seed per plant	M CV	356.8	180.9	268.9	525.0 33.77	376.4 43.31	380.7 37.22
100-seed weight	M(g) CV	4.93	25.97	15.45	12.74 6.41	10.84 13.37	10.49 15.06
Seed shape index	M CV	2.28	1.21	1.75	1.77 8.48	1.73 9.71	1.77 11.98
Plant height	M(cm) CV	138.9	90.8	114.9	143.8 13.63	127.0 15.21	122.5 17.18
No. branches	M CV	14.7	5.6	10.2	18.9 25.29	8.5 39.62	9.9 45.76
Days planting to flowering	M CV	59.7	48.6	54.2	60.0 8.07	50.6 9.38	49.9 9.28
Days flowering to maturity	M CV	68.6	86.5	77.6	76.0 5.04	77.5 6.88	72.8 8.10
Days planting to maturity	M CV	128.3	135.1	131.7	136.0 1.74	127.9 4.31	122.4 5.19

^{*}P $_2,$ P $_c,$ and P $_m$ represent Long 79-4004, Dongnong 33 and value of middle parent, respectively.

^{**}M and CV represent mean and coefficient of variation, respectively.

Effects of genes of some characters in cross Dongnong $33 \times \text{Ji-}50192$ Table 3.

					Effects of ge	genes		
Characters	ш	p	h	į	•	1	X ²	Ъ
% protein	48.31**	* 499 * 0	-5.37	-3.10**	2,69**	3.03	2.23	.250500
Seed wt. per plant	40.56**	18,35**	-26.66	-11.79	-11.62	47.84**	302,39	<.005
No. seed per plant	383.55**	-212,97**	544.67**	10.32	-608.87**	-190.78	1.44	.250500
100-seed weight	6.85**	-12.09**	-2.11	7.02**	5.68**	3.22**	174801.59	.005
Seed shape index	1.7518**	0.4250**	-0.0360	-0.1168**	0.0820	0.0522	8,6012	.010025
Ratio of seeds to stems	0.6668**	0.0161	0.0071	-0.0622**	0.0104	-0.1041**	2,2222	.250500
Plant ht.	131,23**	9.35**	-32,53	-31.08**	0.48	89.10**	3.97	.100250
Diameter of main stem	4.3763**	4.5713**	2.9507	2,4483**	0.2040	-1.4417	1255993.0	.005
No. branches	9.34**	-14.03**	37.22**	10.29	-16.47**	-9.43	2.57	.250500
Days plant- 48,40** ing to flowering	. 48,40**	-8.20**	6.19**	8.40**	-1.13	-0.06	4.06	.100250
Days flow- ering to maturity	76.04**	-18.25**	-24.03	-7.78	6.57*	27.09**	6529.61	<.005
Days planting to maturity	116.22**	10.05**	6.22	8.83**	-16.37**	11,20**	2941.80	<.005

 $^{*,**}\!\!\!^{*,**}\!\!\!^{Significant}$ at the 0.05 and 0.01 possibility level, respectively.

Table 4. Heritability of some characters in F2 generation

	(CI	CI	I ·	CIII	CIV	CV	CVI
Characters	h _n 2*	h _b ^{2*}	h _n	h _b ²				
% protein	63.10	78.40	26.97	72.77	75.06	77.11	62.54	72.26
Seed wt. per plant	14.40	44.72	20.90	23.70	61.41	28.21	38.21	54.39
No. seed per plant	16.71	59.50	18.63	66.63	71.66	62.31	30.44	50.55
100-seed weight	19.85	59.26	26.52	88.68	85.26	87.23	83.39	72.32
Seed shape index	86.74	79.96	57.77	75.46	27.40	45.81	52.85	34.41
Ratio of seeds to stems	48.54	54.29	26.90	55.45	64.87			
Plant height	24.17	52.94	50.83	56.04	85.95	85.34	31.36	56.02
Diameter of main stem	71.32	37.16	32.12	41.21	77.78			
No. branches	52.41	4.77	10.74	45.18	73.38	39.26	39.12	29.52
Days planting to flowering			74.23	94.64	96.48	89.68		66.34
Days flowering to maturity			48.41	75.15	87.89	83.62		88.08
Days planting to maturity	71.75	85.47	73.08	79.45	96.15	95.17	87.73	85.17

 $^{^{\}mbox{\scriptsize h}}_{\mbox{\scriptsize n}}^{\mbox{\scriptsize 2}},~h_{\mbox{\scriptsize b}}^{\mbox{\scriptsize 2}}$ represent narrow-sense and broad-sense heritability, respectively.

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1) Comparative performance of near-isogenic lines for yellow mosaic virus infection in soybean.

The yellow mosaic disease of soybean is a serious disease in the foothills of Uttar Pradesh and other parts in northern India. It is transmitted through white fly (Bemisia tabaci Genn.). Breeding for resistance to yellow mosaic has been one of our breeding objectives (Ram et al., 1985). However, the quantitative assessment of effect of yellow mosaic on the agronomic performance of soybean has not been investigated (Tisselli et al., 1980), particularly using near-isogenic lines. Therefore, an attempt has been made to evaluate the comparative performance of advanced generation breeding lines of soybean derived from F5 generation individual plant progenies and were almost similar except for reaction to yellow mosaic virus.

Materials and methods: Resistant, moderately resistant, and susceptible plants for yellow mosaic virus were selected in rainy season of 1982 from individual plant progeny rows of F₅ generation from different crosses involving resistant sources, i.e., UPSM-534 and *Glycine formosana*. The crosses were UPSM-534 x Clark-63, 'Semmes' x UPSM-534, (T-49 x Lee) x UPSM-534, PS-22 x UPSM-534, T-49 x UPSM-534, UPSM-534 x 'Bragg', (*G. formosana* x Bragg) x Bragg. 'Jupiter' (highly susceptible to yellow mosaic virus) was used as an infector row after each 50 rows.

Next year, single-plant progenies of selected plants were grown in single rows of 3 m length, spaced 60 cm apart with 2 replications in a compact family block design, where crosses were in main plot and progenies (resistant, moderately resistant and susceptible) constituted the subplot. One row of Jupiter was planted after each 10 rows. Observations were recorded for plant height, pods per plant, 100-seed weight, and yield per plant on 10 plants from each replication.

Results and discussion: Plant height, pods per plant, 100-seed weight, and seed yield per plant were adversely affected by yellow mosaic (Table 1). The resistant and moderately resistant categories within a cross were usually similar but superior to the susceptible version. However, in none of the crosses, differences among progenies within a cross were significant. Resistant lines gave higher yield (1.46 to 37.78%) in comparison to susceptible ones and moderately resistant isogenic lines also outyielded by 2.08 to 33.33% the susceptible lines. The reduction in yield to a level of about 30% in the susceptible isogenic lines appears to be quite substantial, but it turned out to be nonsignificant, probably due to wide variation in yield between plants.

We, therefore, feel that, in a selection program for improving soybean yield, the progenies showing mild symptoms of yellow mosaic virus but otherwise looking high-yielding and promising should not be altogether discarded. This preliminary observation needs further confirmation using yield data on plot basis.

Table 1. Performance of different near-isogenic lines for yellow mosaic in soybean $% \left(1\right) =\left(1\right) +\left(1\right)$

Cross		Plant height (cm)	Pods per plant	100-seed weight (g)	Seed yield/ plant (g)	
UPSM-534 x C1-63	R MR S	82.90 87.80 88.10	73.50 74.00 63.80	15.45 15.91 15.05	28.75 29.60 23.35	23.13 26.77
UPSM-534 x Bragg	R MR S	54.30 52.50 57.20	80.80 65.50 59.90	13.75 13.21 12.15	22.50 22.80 19.50	15.38 16.92
PS-22 x UPSM-534	R MR S	87.40 80.80 84.60	59.40 65.60 49.30	15.15 15.00 14.45	24.80 24.00 18.00	37.78 33.33
PS-22 x UPSM-534	R MR S	98.00 102.00 97.30	97.90 106.50 80.20	15.12 15.13 15.00	25.50 24.80 20.50	24.39 20.98
DISOY x UPSM-534	R MR S	88.90 86.10 88.00	83.10 92.10 86.00	15.21 15.62 14.80	28.80 33.50 27.00	6.67 24.07
Semmes x UPSM-534	R MR S	87.50 89.20 85.00	81.80 81.50 70.00	13.25 14.00 13.00	14.50 12.60 12.10	19.83 4.13
(T-49 x Lee) UPSM-534	R MR S	80.70 77.60 79.50	90.50 87.70 92.80	13.96 13.80 13.50	20.80 21.60 20.50	1.46 5.36
(T-49 x Lee) UPSM-534	R MR S	86.00 87.80 87.00	78.00 75.50 75.50	13.85 13.45 13.21	21.50 19.60 19.20	11.98 2.08
(G. formosana x Bragg) x Bragg	R MR S	56.00 54.90 56.20	66.00 72.50 68.80	10.66 10.80 9.65	15.30 15.80 12.50	22.40 26.40
Hardee x Pb.−1	R MR S	84.50 91.90 86.00	107.50 103.30 80.40	13.00 12.91 12.40	25.50 30.00 24.80	2.82 20.96
C.D. 5%		20.36	NS	NS	7.89	
C.V. %		12.32	21.50	4.91	17.58	

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1) Light shock in soybean plantlets?

In a new soybean breeding program, 70 cross combinations were performed between the following varieties: 'Agripro', 'Beeson', 'Caloria', 'Corsoy', 'Extra Early', 'Mikawashima', 'Norman', 'SRF150', 'SRF307 P', 'TXK505', 'TXK535', 'Vansoy', and 'Wells'. Mother plants were the varieties indicated in Table 1; in this table is also reported the number of combinations involving each variety used as female and the number of seeds obtained.

Table 1.	Paculte	of the	croscos	and	their	curviva 1	

Bearing seed variety	No. of combinations	No. of seeds obtained	No. of plantlets survived
Agripro	6	15	0
Beeson	8	22	0
Corsoy	6	15	0
Mikawashima	8	33	30
Norman	7	23	0
SRF150	6	19	6
SRF307 P	6	42	0
TXK505	1	1	0
TXK535	7	33	0
Vansoy	9	36	0
Wells	6	15	0

The 263 F_1 seeds were sown in plastic containers with holes, of diameter of 5 cm and 8 cm deep, on May 30 in a slight shadow greenhouse, randomizing the 70 combinations. Emergence was pretty normal, but on June 15, when the plantlets with open cotyledons were transplanted, most of them showed some discoloration of green tissues and only 33 plants survived few days. They came from crosses between Mikawashima (as mother plant), x Wells, SRF307 P, SRF150, Beeson, Caloria, Corsoy, and TXK505. Also all seedlings of SRF150 x Wells and SRF150 x Beeson survived.

Since no disease symptom was found and the quite normal techniques were applied, we have strong suspicions that light shock and, in second instance, temperature shock, were crucial for most of the plants. Moreover, because of the different mortality rate, we suggest that genetic background is involved.

Thus, Mikawashima, a Japanese variety already mentioned (Olivieri and Parrini, 1983; Parrini and Olivieri, 1984) for having its high unitary seed weight enhanced the plant yield in our breeding programs, seems also endowed with other particular genes.

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1) An interesting nodulation response of soybean cultivars to Bradyrhizobium japonicum strain S32.

Slow-growing Bradyrhizobium japonicum strain S32 formed effective nodules on tap and lateral roots of Glycine max cv. 'Kitamusume'. In contrast, strain S32 formed a few large nodules with pink interior and several small nodule-like structures only on the lateral roots of 'Toyosuzu' cultivars. Although acetylene-reduction activity was detected in the nodules of Toyosuzu, the top of the plant showed no response. A color difference between the two cultivars with S32 was observed 25 days after seeding and inoculating.

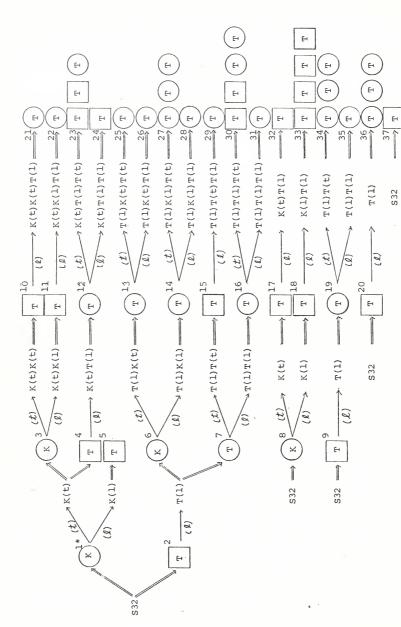
The same response as that shown by Toyosuzu with S32 was observed in 16 among 39 S32-inoculated cultivars. Thirteen of the 16 cultivars that showed ineffective response to inoculation with S32 were resistant to soybean cyst nematode, and the other three were susceptible. On the other hand, 18 of the 23 cultivars that showed effective responses were susceptible to soybean cyst nematode, while the other five were resistant. This suggests that the response of cultivars with S32 is correlated with resistance to soybean cyst nematode (Table 1).

Strain S32 was isolated from a nodule of a Japanese cultivar 'Okuharadaizu' at Nakashibetsu-cho, which is located in the most northeastern district of Japan.

When the bacterium isolated from a large nodule of Toyosuzu inoculated with S32 was reinoculated to Toyosuzu, nodules were formed on both tap and lateral roots. The nodules had pink-red interior and exhibited acetylene-reduction activity. The plant was dark green and healthy in appearance.

To confirm this interesting response, isolation and inoculation tests of S32 were carried out. The results are shown in Figure 1.

- 1. Strain K(t) was effective in Kitamusume (Response reference number 3) but was ineffective in Toyosuzu (4,17). Strain K(l) was ineffective in Toyosuzu (5,18). Strain K(t) and K(l) were isolated from tap and lateral root nodules of Kitamusume with S32, respectively.
 - Strain T(1) was effective in both Kitamusume and Toyosuzu (6,7,19). It was isolated from a lateral root nodule of Toyosuzu with S32.
- 2. Strain K(t)K(t) and K(t)K(l) were ineffective in Toyosuzu (10,11). The former was isolated from a tap root nodule of Kitamusume with strain K(t) and the latter from a nodule of a lateral root of Kitamusume with strain K(l). On the other hand, strain K(t)T(l) was effective in Toyosuzu (12). Strain K(t)T(l) was isolated from a lateral root nodule in Toyosuzu with strain K(t).
- 3. Both strain T(q)K(t) and T(1)K(1) were effective in Toyosuzu (13,14). These strains were isolated from tap and lateral root nodules of Kitamusume with strain T(1).
 - Strain T(1)T(t) was ineffective in Toyosuzu (15) but strain T(1)T(1) was effective in Toyosuzu (16,35). The former strain was isolated



of two soybean cultivars, Toyosuzu and Schematic diagram in nodulation response Kitamusume, inoculated with S32. Fig. 1.

|=Ineffective response, *=Response reference = Liffective response, K=Kitamusume, (number.

Nodule number, nodule dry weight, acetylene-reduction rate and color of top in soybean cultivars inoculated with strain S32 Table 1.

	Nodule	Nodule number	Nodu	Nodule dry	Acetyle	Acetylene reduction	100	Resistance
Cultivars	Tap	Lateral root	Tap	Lateral	Per	Per gram of of nodule	of top	soybean cyst nematodes
Effective								
Kitamusume	26	99	106	141	29.9	63.6	‡	Sp
Kitakomachi	21	33	181	69	7.3	29.2	‡	S
Tokachi-nagaha	13	47	102	84	12.2	66.1	‡	S
Lee	16	62	122	91	17.1	80.8	‡	S
Custer	10	38	108	101	9.6	45.8	‡	Ж
Ineffective								
Toyosuzu	0	9	0	20	1.3	63.4	ı	×
Suzuhime	0	5	0	30	9.0	18.6	ı	M
Kitakomachi	0	4	0	14	1.3	30.4	ı	S
Peking	0	5	0	11	0.3	25.1	ı	×
Pickett 71	0	2	0	7.5	1.3	16.8	ı	В

 $^{\mathrm{a}}$ +++ indicates dark green leaves, - indicates yellow or light green leaves.

bS = susceptible, R = resistant.

from a tap root nodule of Toyosuzu with strain T(1) and the latter from a lateral root nodule of Toyosuzu with strain T(1).

4. The following eight strains were also found effective in Toyosuzu, namely: K(t)K(t)T(1), K(t)K(1)T(1), T(1)K(t)T(t), T(1)K(t)T(1), T(1)K(1)T(t), T(1)K(1)T(1), T(1)T(1)T(1) and T(1)T(1)T(1). Strains K(t)T(1)T(1), K(t)T(1) and K(1)T(1) were ineffective in Toyosuzu.

These results suggest that strain S32 forms effective nodules on Toyosuzu when the strain is isolated from a lateral root nodule of Toyosuzu that has been already inoculated with S32.

We are preparing a genetic analysis of this nodulation response between Toyosuzu and Kitamusume with S32, and molecular analysis of plasmids of bacteria isolated from lateral root nodules of Toyosuzu inoculated with S32.

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1) Performance of near-isogenic lines lacking seed lipoxygenases.

Normal soybean seeds contain at least three lipoxygenase isozymes, called L-1, L-2, and L-3. These isozymes are responsible for the generation of objectionable grassy beany and green flavors which build a barrier against the wide utilization of soy protein products. Recently, the three types of mutants lacking L-1 (Hildebrand and Hymowitz, 1981), L-2 (Kitamura, 1984), and L-3 (Kitamura et al., 1983), respectively, were found. Genetic studies have demonstrated that the absence of L-1 (Hildebrand and Hymowitz, 1982), L-2 (Kitamura et al., 1985; Davies and Nielsen, 1986), and L-3 (Kitamura et al., 1983) from the seeds is under the control of single recessive alleles, lx_1 , lx_2 , and lx_3 , respectively. By the use of these recessive alleles, we have been attempting to breed soybeans with low levels of the objectionable flavors. So far, the two types of double mutants that lack both L-1 and L-3, and both L-2 and L-3 have been identified. No physiological problems have been observed during the life cycles of the two double mutant soybeans.

In this paper, we report the results of average performance on some characters of the near-isogenic lines in 'Suzuyutaka' lacking the seed lipoxygenases.

Materials and methods: Three to four backcrosses were made to the recurrent parent Suzuyutaka, a leading variety in Tohoku district, with selection of the absence of the isozymes by the improved SDS-PAGE (Kitamura, 1984) to obtain the near-isogenic lines lacking L-1, L-2, and L-3, respectively. BC4F2 seeds lacking L-1·L-3 were obtained from a cross between BC4F2 plants lacking L-1 and L-3, respectively. BC3·4F2 seeds lacking L-2·L-3 were obtained from a cross between a BC3F2 plant lacking L-2 and a BC4F2 plant lacking L-3. BC4F2-derived lines in BC4F3 lacking L-1, L-3, and L-1·L-3, respectively, a BC3·F2-derived line in BC3F3 lacking L-2, and a BC3·4F2-derived line in BC3·4F3 lacking L-2·L-3 were grown in 15-cm inter-plant-space in 2 m rows, 80 cm apart with two replications in Morioka (latitude 40°N), Iwate and Tsukuba (latitude 36°N), Ibaraki, Japan, in 1986. Data were obtained for characters from random 20 plants. Percent protein and percent oil were estimated by NIR analyzer.

Results and discussion: The results of average performance on the characters evaluated in Morioka and Tsukuba are presented in Tables 1 and 2, respectively. The characters were the flowering day, stem height, the number of pods per plant, the number of seeds per plant, seed yield per plant and 100-seed weight. The maturing day, percent protein, and percent oil for the soybeans were evaluated only in Morioka. Although relatively large differences were observed in the number of pods per plant, the number of seeds per plant, and seed yield per plant between two replicates, the analysis of variance showed no significant difference at 5% level of these characters among the lines both in Morioka and Tsukuba. There were no significant physiological and morphological differences through each stage of germination, early growth, flowering, and seed development among the lines. Moreover, no

Performance of some important characters of Suzuyutaka and the near-isogenic lines lacking seed lipoxygenases (average of two replicates, Morioka, 1986) Table 1.

Variety/ line	Flowering	Maturing day	Stem height (cm)	No. pods/ plant	No. seeds/ plant	Seed yield per plant (g)	l 100-seed weight (g)	Protein (%)	0i1 (%)
Suzuyutaka	Aug. 9	Oct. 15	87.2	87.2 79.1	162.9	39.16	24.04	40.55	20.48
L-1-less	Aug. 11	Oct. 22	5.96	80.4	171.5	40.54	23.64	42.00	19.33
L-2-less	Aug. 10	Oct. 22	98.5	81.8	173.0	39.29	22.71	39.63	20.53
L-3-less	Aug. 9	Oct. 15	94.5	77.5	159.1	33.81	21.25	40.50	20.33
L-1.3-less	Aug. 12	Oct. 19	95.7	76.0	159.9	37.21	23.27	42.35	19.68
L-2.3-less	Aug. 9	Oct. 14	9.98	79.3	164.6	37.13	22.56	41.13	20.25

Performance of some important characters of Suzuyutaka and the near-isogenic lines lacking seed lipoxygenases (average of two replicates, Tsukuba, 1986) Table 2.

Variety/ line	Flowering day	Stem height (cm)	No. pods per plant	No. seeds per plant	Seed yield per plant (g)	100-seed weight (g)
Suzuyutaka	Aug. 1	50.2	98.2	178.4	47.81	26.80
L-1-less	Aug. 9	8.65	6.86	189.3	52.84	27.91
L-2-less	Aug. 9	66.2	94.2	175.6	45.83	26.10
L-3-less	Aug. 2	48.2	6.06	173.7	45.41	26.14
L-1.3-1ess	Aug. 9	0.49	117.5	219.7	61.66	28.07
L-2.3-less	Aug. 2	41.9	93.1	179.4	45.62	25.43

difference in seed damage by insect pests, such as pod borers or stink bugs, was observed among the lines in both of the field trials. In another experiment, it was shown that there was no significant difference in activity levels of leaf and young pod lipoxygenases that have been thought to play physiologically important roles among Suzuyutaka and the near-isogenic lines lacking the seed lipoxygenases.

These results indicate that it is possible to breed commercial soybean varieties with low levels of the undesirable lipoxygenase action by the use of the three recessive alleles, lx_1 , lx_2 , and lx_3 . Although neither double mutant seeds lacking both L-l and L-2 nor triple mutant seeds lacking all the isozymes were identified because of the tight linkage between lx_1 and lx_2 loci (Kikuchi and Kitamura, 1985; Davies and Nielsen, 1986), the level of the objectionable flavors of the seeds lacking L-3 and L-2, the latter the most responsible for the generation of n-hexanal (one of the major elements of the flavors) (Matoba et al., 1985) is so low that the double mutant soybeans can be accepted by the soybean food industry.

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1) Variability for some quantitative traits in soybean.

Abstract: Studies on variability in soybean were taken up to work out the magnitude of genetic variability, heritability, and genetic advance among 36 varieties of soybean. Considerable genetic variability was observed for pods per plant, plant height, and grain yield per plant. High heritability and genetic advance were recorded for number of pods per plant and branches per plant. Thus, yield could be considerably improved through intensive selection pressure based on number of pods per plant and branches per plant.

<u>Introduction</u>: Soybean is one of the most important protein and oil rich crops in the world. More than 30% of the edible vegetable oil in the world is extracted from this crop alone. In Pakistan, its cultivation is still in introductory stages, with an average yield of 416 kg/ha (Anon., 1982), whereas its yield potential is very high (Chaudhry et al., 1984). To exploit full yield potential, estimation of genetic variability components is essential. The present study, therefore, was undertaken with a view to obtain information on the extent of genetic variability in respect to important agronomic characters in 36 cultivars of soybean available in our germplasm pool.

Materials and methods: Thirty-six soybean varieties of diverse origin were grown in a randomized complete block design with four replications at Experimental Farm AEARC, Tandojam. Each replication consisted of a single row 4 meters long. Seed was drilled at a row distance of 45 cm. Plant-to-plant distance was maintained at 5 cm by thinning the crop before first irrigation. At maturity, five competitive plants from each replication of all the varieties were randomly selected and the observations were recorded for plant height, number of branches per plant, pod length, pods per plant, seeds per pod, 100-grain weight and grain yield per plant.

Statistical analysis was performed and genetic selection parameters were computed according to Johnson et al. (1955).

Results and discussion: The estimates of genetic parameters [genotypic variance $(\sigma^2 g)$, phenotypic variance $(\sigma^2 p)$, heritability (h^2) and expected genetic advance (GS)] are presented in Table 1. The estimate of variance due to genotypic and phenotypic effects varied widely for different characters. The highest genotypic and phenotypic variance was recorded for number of pods/plant (1163.27 and 1240.74) which was followed by plant height (275.84 and 383.43), grain yield/plant (41.46 and 67.76), moderate for 100-grain weight (6.06 and 6.44). Low variability was observed for branches/plant (1.52 and 1.61), seeds/pod (0.07 and 0.08) and pod length (0.06 and 0.06). The extent of phenotypic coefficient of variation (PCV) was slightly higher than genotypic coefficient of variation (GCV) for all the traits under study. Very little difference between corresponding phenotypic and genotypic variability of traits studied suggest that environment had negligible effect on these characters. Hence, selection for these characters will provide a greater chance of genetic improvement.

A high GCV value for the characters would appear reliable if substantiated by the high heritability estimates. The heritability values ranged from

Table 1. Genetic parameters for various quantitative traits in soybean

Characters	Ι×	Genotypic variance	Genotypic coefficien Phenotypic of vari- variance ability	Genotypic coefficient of variability	Phenotypic coefficient of variability	Herit- ability	Genetic advance	Genetic advance % of mean
Plant height (cm)	68.66	275.84	383.43	24.19	28.52	71.94	29.02	42.27
Pod length (cm)	3.89	90.0	90.0	6.31	6.55	92.89	0.49	12.53
Seeds/pod	2.11	0.07	0.08	12.49	13.45	86.26	0.50	23.91
Branches/plant	2.62	1.52	1.61	90.74	48.46	94.32	2.47	94.16
Pods/plant	64.73	1163.27	1240.74	52.69	54.42	93.76	68.03	105.10
100-grain weight (gm)	14.77	90*9	77.9	16.67	17.18	94.17	4.92	33,33
Grain yield/ plant (gm)	16.51	41.46	67.76	39.00	49.86	61.19	10.38	62.85

61.19 to 94.43%. The branches/plant had the highest estimate followed by 100-grain weight, pods/plant, pod length, seeds/pod, plant height and grain yield/plant. In the present investigation, pods and branches per plant showed the highest values for GCV as well as heritability, thus offering a greater scope for genetic improvement using them as selection criteria. Similar observations for pods/plant have earlier been reported by Malik et al. (1983) in mungbean and Nararajan and Arumugam (1979), Seth et al. (1972) and Sharma et al. (1977) in French beans.

Genetic advance, expressed as percent of mean, was highest for pods/plant (105.10) followed by branches/plant (94.16), grain yield/plant (62.85), plant height (42.27) and lowest was for pod length (12.53). A high genetic advance accompanied with high heritability estimate offers a most effective criterion of selection (Johnson et al., 1955; Panse, 1957). In the present study, high estimates of genetic advance accompanied with high heritability and genotypic coefficient of variation for number of pods/plant, branches/plant and grain yield/plant led to high expected genetic gain and suggest the positive role of these characters in genetic improvement of soybean through direct mass selection. Vinaya Rai et al. (1981) in Sesamum indicum and Khalid et al. (1984) in mungbean have also reported high heritability and genetic advance for branches/plant, and suggested that selection for number of branches/plant would be rewarding in breeding for high yield. The higher heritability and comparatively lower estimates of genetic advance for pod length and seeds/pod indicate a relatively poor role of these characters in helping a substantial genetic gain. Panse (1957) has reported that, if heritability is mainly due to the nonadditive genetic effects, the genetic gain would be low, whereas, if heritability is due to the additive gene effects, a high genetic advance may be expected.

The present study reveals that pods per plant and branches per plant exhibited high heritability, genetic advance and genetic coefficient of variance and form the most reliable indices of selection for genetic improvement in soybean.

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M. A. Rajput Ghulam Sarwar K. H. Tahir

2) Induction of polygenic variance in soybean.

<u>Abstract</u>: The effect of gamma-rays on variability for three quantitative characters (number of pods, seeds, and grain yield per plant) of soybean were studied. In the M_2 generation, the shifts of mean values of irradiated material for all the polygenic characters occurred towards the positive or negative direction. However, the coefficient of variability increased in all the treatments for the attributes studied. This additional genetic variability would provide an opportunity for improvement of soybean by selection.

<u>Introduction</u>: Soybean, a crop of high nutritive value (444 cal/100 g), is also a most prospective oil crop for Pakistan. Soybean is ideally suited for mutation breeding with a high probability of altering and improving any morphological character through induced mutations (Rajput and Siddiqui, 1983; Zakri et al., 1982). Hence, the present study was undertaken to measure the magnitude of variability induced by different doses of gamma-rays in soybean.

Materials and methods: Air-dried seeds of three varieties ('Loppa', T-15 and 'Columbus') were exposed to different doses of gamma-rays (0, 10, 15, 20 and 25 kR). Irradiated and control seeds were planted in the field to grow the $\rm M_1$ generation. To study the variability in the $\rm M_2$ generation, the seed was prepared by drawing first formed 10 pods of normal looking 150 $\rm M_1$ plants from each variety/treatment. The trial was planted in a randomized complete block design with four replications. At flowering stage, 20 plants per replication were tagged at random and various observations on developmental and productive attributes were recorded. In this paper, results of M2 studies regarding the effects of acute gamma-irradiation of soybean seeds on primary productive traits are discussed.

Results and discussion: The data on mean, range, and variance for yield and yield components (pods per plant, seeds, and grain yield per plant) are given in Tables 1-3.

In M2 generation, substantial changes were noted in mean values of all the treatments of all the genotypes when compared with their respective controls. Pods per plant increased along with substantial increase in coefficient of variability. This increased number of pods accompanied with enlarged variability provided opportunity for selecting heavy bearing plants (Rajput and Siddiqui, 1983).

The depressive effects of gamma-rays on mean values for seeds per plant and grain yield per plant could be due to the occurrence of polygenic mutations mostly in negative direction. However, the variability was sufficiently enlarged, which could be exploited for selecting desirable plant types. Similar detrimental effects of radiation along with increased variability have already been reported by many workers (Bhattia and Swaminathan, 1962; Ghafoor et al., 1968; Rajput, 1974).

Table 1. Range, mean, standard deviation and coefficient of variability for number of pods per plant in M_2 generation of soybean

Variety	Dose	Mean	Range	S.D.	C.V. (%)
Columbus	0KR	54.4	30-110	19.0	34.9
OO I amb ab	10KR	55.1	18-89	15.4	27.9
	15KR	61.3	18-129	30.7	50.1
	20KR	65.3	32-110	21.8	33.3
	25KR	59.1	15-123	24.1	40.7
Loppa	0KR	66.6	22-102	19.2	28.8
••	10KR	63.8	29-120	21.8	34.1
	15KR	67.5	27-132	20.9	31.1
	20KR	70.9	22-162	25.3	35.7
	25KR	67.2	29-118	20.9	31.3
T-15	0KR	72.4	36-110	18.5	25.6
	10KR	83.8	22-150	29.8	35.5
	15KR	85.6	22-170	30.9	36.1
	20KR	84.3	29-139	26.4	31.4
	25KR	77.4	31-153	29.9	38.5

Table 2. Range, mean, standard deviation and coefficient of variability for seeds per plant in $\ensuremath{\text{M}}_2$ generation of soybean

Variety	Dose	Mean	Range	S.D.	C.V. (%)
Columbus	0KR	118.0	48-196	41.1	34.9
	10KR	113.9	56-225	37.1	32.6
	15KR	100.9	29-246	43.7	43.2
	20KR	101.6	37-224	33.9	33.3
	25KR	94.2	33-259	44.3	47.0
Loppa	0KR	129.2	52-238	38.7	29.9
	10KR	103.2	34-247	46.3	44.8
	15KR	100.9	22-261	47.1	46.7
	20KR	99.8	41-253	35.7	35.8
	25KR	88.8	29-281	52.3	58.8
T-15	0KR	163.7	74-279	55.5	33.8
T-15	10KR	150.0	59-268	52.1	34.7
	15KR	139.9	72-254	37.5	33.9
	20KR	139.2	51-348	62.2	44.7
	25KR	125.8	29-284	62.0	49.3

Table 3. Range, mean, standard deviation and coefficient of variability for grain yield per plant in M_2 generation of soybean

Variety	Dose	Mean (gm)	Range (gm)	S.D.	C.V. (%)
Columbus	0KR	20.4	5.81-48.21	9.9	48.4
	10KR	18.6	4.94-40.0	9.9	53.7
	15KR	18.6	4.79-63.03	10.8	58.0
	20KR	18.3	9.03-63.89	8.9	48.2
	25KR	18.5	3.58-55.26	8.9	48.5
Loppa	0KR	16.8	6.72-32.00	6.1	35.9
1.1	10KR	12.6	4.95-30.75	6.1	48.1
	15KR	12.6	2.19-26.00	5.5	43.3
	20KR	12.2	4.90-38.22	6.8	55.7
	25KR	11.5	3.24-36.55	6.8	58.7
T-15	0KR	25.9	7.70-66.25	12.9	49.9
	10KR	23.9	6.99-80.72	14.5	60.9
	15KR	20.9	5.92-35.00	7.9	37.6
	20KR	21.2	7.0-45.34	11.2	52.9
	25KR	19.6	4.93-48.26	10.1	55.3

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1) Phenotypic stability of soybean in Peru.

Introduction: In countries like Peru, where soybean is an introduced crop, it is necessary to utilize high technology suitable for the coast and jungle ecological conditions, in order to achieve productive success. Besides mechanization, it is very important to use seed of high genetic value, stored under optimum conditions. These two conditions would contribute to minimize the risks that very often affect the farmers. The basic idea is to consider the variety to be used and its possible interaction with the environment.

There are two ways for evaluating the adaptive capability of a species; the first, related to survival, commonly used by the evolutionists, and the second, one which considers the stability and consistency of performance, mostly used by plant breeders.

There are several methods of stability evaluations in use; the earliest one was described by Finlay and Wilkinson (1963). Eberhart and Russell (1966) modified the previously indicated interpretation because, in general, the hybrids having regression coefficients smaller than "l" had productive performances below the general average. They established that a variety is stable if it has a regression coefficient equal to "l", a high average, and the regression variance of deviation should be small or equal to "0".

Materials and methods: The study involved the results of several soybean varieties trials conducted from 1975 to 1976 at five locations in Peru. The locations (Coordinates 5° to 9° South Latitude) were in the jungle: Huarangopampa, El Porvenir, Pucacaca and Tulumayo; and Mallares in the Northern Coast.

Trials were set as randomized block design (at five locations). The plots consisted of four 5 m-long furrows, separated 0.60 m and planted in a density of 400,000 to 450,000 plants per ha. The performance was evaluated using the two central furrows. Data of plant number, blooming date, and seed production were recorded and analyzed. Previous to planting, the seeds were inoculated with Nitragin "S".

The studied varieties were: 'Jupiter', 'Hampton 266a', 'Hardee', 'Improved Pelican', 'Cobb', 'Bossier', 'Davis', 'Tracy', 'Forrest', 'Columbus', 'Clark 63', 'Woodworth', and 'Williams'.

The method used in the calculations and the interpretation was that of Eberhart and Russell (1966), which is a modification of Finlay and Wilkinson (1963) methodology. The statistical model used for defining the stability parameters was:

$$Y_{ij} = M_i + \beta_i I_j + \sigma_{ij}$$

where:

- Y_{ij} = Average performance of i^{th} variety under the j^{th} environment (i = 1, 2, ... v) and (j = 1, 2, ... n);
- M_{\star} = Mean of ith variety under all the environments;
- β_i = Regression coefficient which measures the response of ith variety to the different environments;
- σ_{ij} = Regression deviation of i^{th} variety under the j^{th} environment;
- I = Environmental index at j th environment, defined in terms of environment deviation from the mean of all environments.

Results and discussion: A Barttlett test confirmed homogeneity of variance among varieties; therefore, the error mean squares were considered as estimators of the population variance. Also, the analyzed responses were independent of density and there was no need of data adjusting.

Table 1 shows the 13 varieties' average responses and their environmental index for the five locations.

The average production of these 13 varieties ranged between 1,754 and 3,101 kg/ha; these extremes corresponded to Woodworth and Improved Pelican, respectively.

The environmental average for the five localities ranged from 1,706 kg/ha to 2,479 kg/ha for plots at Pucacaca (Jaen Province) and Sullana locations, respectively. Environmental indexes ranged from -0.325 at Pucacaca location to 0.139 at Sullana.

Table 2 presents the variance analysis showing high significant difference among varieties and influence of variety x location interaction (linear). The interaction can be interpreted as the existence of genetic differences among varieties according to their regression on the environmental indexes.

Table 3 presents the stability parameters for the 13 soybean varieties. The better varieties for yield under the five environments were Improved Pelican and Jupiter. In a second order group were Forrest, Davis, and Hardee.

The regression coefficient reached values between -1.09 and 2.50 for Clark 63 and Jupiter, respectively. The wide range of variation allowed the Clark 63 variety to show a slope of the regression line highly significant and different to 1.

The regression deviation indicative of the degree of consistency to environmental changes showed values between -0.018 and 0.148 for Clark 63 and Cobb varieties, respectively. The Jupiter and Cobb varieties showed highly significant differences while the Forrest, Davis, and Bossier were only significantly different of zero. That suggests that the significant variety-environment interactions could be due to lack of consistency in response of some of the varieties to the different environmental conditions.

Table 1. Average yield of 13 soybean varieties tested at five different locations

		Н	Production in	kg			
Variety		Huaran-	El			Average	age
	Sullana	gopampa	Porvenir	Pucacaca	Tulumayo	kg/plot	kg/ha
Jupiter	2.051	2.342	1.540	1.051	2.189	1.835	3,058
Hampton 266-A	1.442	1.372	1.252	0.932	1.146	1.229	2,048
Hardee	1.713	1.770	1.379	1.145	1.336	1.469	2,448
Improved Pelican	2.226	1.772	1.971	1.222	2.110	1.860	3,101
Cobb	1.150	1.820	1.106	0.810	0.881	1.153	1,922
Bossier	1.030	1.076	1.204	0.805	1.559	1.135	1,891
Davis	2.056	1.409	1.394	0.854	1.722	1.487	2,478
Tracy	1.039	1.172	1.280	0.900	1.184	1.115	1,858
Forrest	1.936	1.304	1.717	1.139	1.516	1.522	2,537
Columbus	1.404	1,407	1.375	1.084	1.259	1.306	2,176
Clark-63	0.965	1.148	1.078	1.501	1.051	1.149	1,914
Woodworth	1.086	0.929	1.086	0.892	1.269	1.052	1,754
Williams	1.244	1.044	1.370	0.972	1.469	1.220	2,033
Average	1.488	1.428	1.365	1.024	1.438	1.349	2,248
Environmental Index	0.139	0.079	0.016	-0.325	0.089		

Table 2. Variance analysis used for estimation of stability in the performance of 13 soybean varieties

Source of variation	df	SS	MS	F
Total	64	9.189		
Varieties (V)	12	2.303	0.358	7.458**
Environments (E)	52	4.886		
V×E				
- Environmental (linear)	1	1.745		
- Var. x Envir. (linear)	12	1.279	0.106	2.208*
- Pooled deviations	39	1.862	0.048	
Error	180		0.080	

Table 3. Average yield kg/ha, b and S^2d stability parameters and classification of 13 soybean varieties

Duncan Classification +						sification ⁺
Varieties	Yield	test	b _i	s ² di	Group	Description
Imp. Pelican	3,101	а	1.95	0.011	2	Stable
Jupiter	3,058	а	2.50	0.067**	1	Generally good re- sponse, nonconsistent
Forrest	2,537	Ъ	1.18	0.050*	1	Generally good re- sponse, nonconsistent
Davis .	2,478	bc	2.12	0.036*	1	Generally good re- sponse, nonconsistent
Hardee	2,448	bc	1.05	0.022	2	Stable
Columbus	2,176	cd	0.63	-0.013	2	Stable
Hampton 266-A	2,048	de	0.92	-0.005	2	Stable
Williams	2,033	de	0.66	0.019	2	Stable
Cobb	1,922	de	0.98	0.148**	1	Generally good re- sponse, nonconsistent
Clark 63	1,914	de	-1.09**	-0.018	3	Better response under unfavorable environ- ment, consistent
Bossier	1,891	de	0.89	0.045*	1	Generally good re- sponse, nonconsistent
Tracy	1,858	de	0.50	-0.003	2	Stable
Woodworth	1,754	_ e _	_0.46 _	0.000	_ 2	Stable
General aver.	2,248					

⁺According to stability parameters.

According to Eberhart and Russell (1966) methodology, varieties showing regression coefficients close to one and deviations near to zero are ideal. These characteristics added to performances above the general average allow us to draw some conclusions; however, the general average is useless because the majority of these varieties have no optimum adaptive advantage in terms of performance. Moreover, they are varieties introduced from other environments and selected to perform in environments quite different to that of our country. Therefore, the ideal varieties for our conditions would be Hardee and Improved Pelican.

The classification and description reported in Table 3 give some orientation; however, its confirmation requires testing a wider range of environments.

Conclusion: In general, selection is made considering those traits indicative of vigor that are highly correlated to productive performance. The use of stability should make that criteria change. What is necessary are stable varieties, or varieties having average stability, instead of varieties having high performance but low stability.

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1) Double-crop soybean production techniques in Turkey.

Turkey is located between 36° and 42° N latitude. Syria, Iraq, and the Mediterranean Sea border Turkey on the south; the Black Sea on the north; Russia and Iran on the east; and the Aegean Sea, Greece and Bulgaria on the west.

The country has a total area of 77.9 million hectares. Approximately 36% is cultivated, 26% is pasture and meadow land, and the remaining 38% is forest or unproductive land (Tarimsal Yapi ve üretim, 1984).

Because of the suitable climate and soil conditions, it is possible to get more than one crop in a year in Aegean, Mediterranean, and southeast Anatolian regions of Turkey. Following the cereal harvest, soybean can be grown successfully as a second crop in the summer season. These regions have the most productive land of Turkey. Approximately 38% of the agricultural lands are irrigated with different sources of water. The main crops in the area are cotton and wheat.

After 10 years of research and four years of production observation, it is concluded that soybean can successfully be grown as a second crop with a satisfactory yield in these regions.

Soybean production possibilities as a part of a double-cropping system: In the Aegean, Mediterranean, and southeast Anatolian regions of Turkey, there are 1.3 million hectares of irrigated land. Most of these lands are used every year for cotton growing or cotton + wheat rotation. In the cotton + wheat growing system, wheat and barley are harvested from the end of May to mid-June. The fields are then left unplanted until cotton planting time (until next April).

In the southern and southeastern part of Turkey, wheat and barley farming is done on irrigated areas, approximately 303,000 hectares, Only about 20% of this land is used for double-cropping and 80% is left unplanted until the next season. Thus, 243,000 hectares of potential land is available for soybean farming. After wheat or barley harvesting, soybean can successfully be grown within 115-135 days in summer time (6lez et al., 1981).

According to preliminary studies, there are around 300,000 hectares of land suitable for soybean farming in Turkey. This area is expected to reach 500,000 hectares under the new irrigation projects.

The present status of double-cropped soybean production in Turkey: Soybean farming first began in Adana, Hatay, and içel provinces of Turkey in 1975, and in the following years spread out to K.Maras, G.Antep, Ş.Urfa, Diyarbakir, Antalya, Mugla, Aydin, Manisa, and Izmir provinces.

Soybean production in Aegean, Mediterranean, and Southeast Anatolian regions is given below (Tarimsal Yapi ve "uretim", 1984).

Table 1.	Soybean	production	in	Aegean,	Mediterranean,	and	southeast	Anatolian
	regions	of Turkey						

Year	Area (ha)	Production (ton)	Yield (kg/ha)	Max. yield (kg/ha)
1980	122	180	1019	1500-2000
1981	17,000	15,000	882	1500-2000
1982	24,300	40,000	1650	2500-3000
1983	23,000	44,000	2170	3000-3500
1984	28,000	78,000	2780	4000-4500
1985	50,000	110,000	2200	4500-5000

As can be seen, the annual soybean production was 180 tons in 1980, then in 1985 it increased to 110,000 tons. Also, the yield per hectare increased from 1019 kg/ha to 2200 kg/ha in 1980 and 1985, respectively. The maximum yield was 1500-2000 kg/ha in 1980 and it also increased to 4000-4500 kg/ha in 1985. More than 90% of the total planted areas were double-cropped.

Soil preparation: In the double-crop soybean growing area, wheat is harvested from late-May to the middle of June. The wheat straw is burned before tilling on approximately 50% of the double-crop acreage. Usually, extremely dry soil conditions that existed after wheat harvest make it necessary to irrigate prior to seedbed preparation. Seedbed preparation consists of chisel plowing and then disking or of a two-diskings operation. After the final disking, a "cultipacker" type implement is used to level and firm the soil. Less frequently, farmers prepare the soil for planting and then irrigate after planting.

At this time, Turkish researchers are investigating the use of minimum tillage as a double-crop soybean production tool, but the necessary equipment is not currently available to the farming public.

<u>Inoculation and fertilization</u>: Soybean is a new crop for the Aegean, Mediterranean, and Southeast Anatolian regions of Turkey. Since the soil does not contain sufficient *Rhizobium japonicum* at planting time, seed inoculum must be used. The majority of this inoculum must be imported because of insufficient production in Turkey.

For double-crop soybean production, 40 kg of N and 60--80 kg P_2O_5 per hectare are used. This fertilizer is usually broadcast and then disked in prior to planting. Sometimes farmers apply an additional 20--40 kg N per hectare just before the first irrigation (full-bloom stage).

The Turkey soils are high in potassium; therefore K_2O fertilization is not necessary. In Turkey, the soil pH ranges from 6.5 to 7.5. Even though iron chlorosis has been observed on some high alkaline soils, the addition of iron fertilizer is uncommon.

Soybean varieties and planting: The growing season available for double-crop soybeans ranges from 115-135 days, depending on location. For this reason, Maturity Groups II, III, and IV varieties are grown. Widely used varieties include: Amsoy 71, Calland, A 3127, A 2575, Mitchell, Mitchell 450, Mitchell 410, L 4106 and L 4207. Mitchell 450 and L 4106 also may be used successfully as double-crop varieties if planted no later than June 20. Amsoy 71 and A 2575 are the only varieties that can be successfully grown after July 1.

Double-crop soybeans are typically planted from June 1 to July 7. Planting soybeans after July 7 is not recommended. Soybeans planted after this date suffer severe yield reductions and cause problems with harvest and drying.

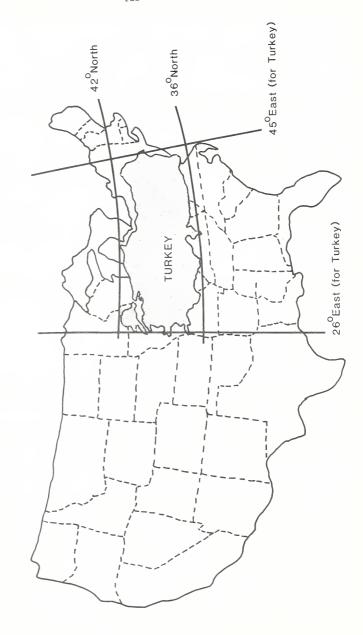
Soybeans are planted at an intra-row distance of 2 to 4 cm in 40 to 70 cm rows depending on variety and time of planting. Row widths of 60 to 70 cm are common in early plantings, while narrower row widths (40 to 50 cm) are used in later plantings. The planting depth is 4 to 5 cm depending on soil moisture.

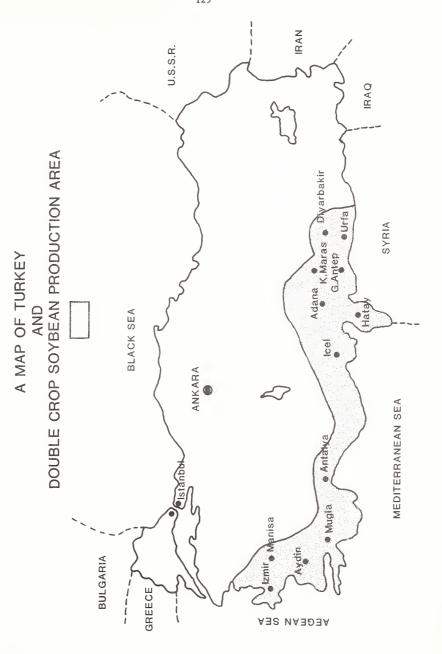
Cotton planters are commonly used to plant soybeans in Turkey. This results in many cracked and otherwise damaged seeds. Seeding rates range from 70 to 120~kg/ha depending upon the row width. No insecticides or fungicides are used at the time of planting.

Table 2. Performance of registered double-crop soybean varieties at four locations in Turkey in between 1976 and 1984 (Arastirma özetleri, 1984)

		Days to	maturit	у	Yield (kg/ha)			
Varieties	Adana	Antalya	Izmir	Diyar- bakir	Adana	Antalya	Izmir	Diyar- bakir
Amsoy 71	97	95	97	103	3790	3473	3610	1593
Calland	110	106	113	109	3205	3433	3535	1533
Mitchell 450	120	120	-	109	3100	3915	-	1500
L 4106	111	106	-	93	3917	3330	-	1040
Mitchell	110	106	117	110	2630	3557	3435	1407
Mitchell 410	110	110	-	-	3400	3370	-	-
Washington V	100	101	108	103	3110	4063	3245	1736
Shawnee II	97	97	99	98	3337	3817	3595	1760
A 3127	110	-	-	-	3620	-	-	-
A 2575	100	-	-	-	3140	-	-	-
Latitude 37	°00'N	36°52'N	38°26'N	37°56'	N			
Elevation	50 m	42 m	25 m	660 m				

TURKEY'S MAP SUPERIMPOSED ON THE U.S.A. MAP





Weed and insect control: Virtually all double-crop soybeans are grown under irrigation. Many weed species fluorish under these irrigated conditions. Most common weed species include: common lambsquarters, common purslane, Virginia buttonweed, Bermudagrass, green foxtail, broadleaf signalgrass, Johnsongrass, large crabgrass, barnyard grass and nutgrass. Generally, herbicides are not used, but some farmers use triflourin.

Soybeans planted in 60- to 70-cm rows are cultivated by tractor; however, narrower row widths must be cultivated by hand. Soybeans are typically cultivated 2 or 3 times during the growing season.

The major insect pest problem of Turkish double-crop soybean is the white fly (Bemesia tabaci). This is, however, not a serious problem, since all recommended varieties have white-fly resistance. In spite of varietal resistance, damage can occur in extremely late-planted soybeans due to the migration of white flies from the maturing cotton. In this situation, one or two applications of insecticide are used for control.

Bollworm, green stinkbug, corn leafworm, and two-spotted spiders also affect soybeans to a limited extent. One insecticide application is usually sufficient for control of these pests.

<u>Irrigation</u>: Double-crop soybeans are grown only under irrigation in Turkey. Soybean production without irrigation is not recommended, due to the extremely hot and dry growing season. Depending on location, three or four irrigations are used. The first irrigation is at full bloom and subsequent irrigations are at 15-day intervals up to 15 days prior to harvest. Omission of the last results in yield reduction of 20-25%.

The flat topography of the soybean growing area allows for the use of furrow irrigation. Sprinkler irrigation is uncommon.

<u>Harvesting and drying</u>: Although it varies according to planting times and varieties, double-crop soybean is mostly harvested from the end of September through October. In Turkey, soybean is harvested with conventional combines. For this reason, the seed lost during harvest is up to 15-20%.

Humidity in the harvested material is about 13-15%, and, if the seed moisture content is higher than 95%, the seeds are dried under natural conditions. In the autumn, it may be a problem if the rain comes early for lateplanted soybeans.

Storing, marketing, and processing: After harvesting, dried soybeans are stored in ordinary storage. In Turkey, there are not any modern soybean facilities.

In Turkey, the soybean support price is announced by the government every year before planting time with guaranteed buying. The farmers sell their soybeans to government or private companies as soon as possible after harvesting, because most of the farmers have not any modern soybean storage. The soybean price in 1985 was 26 cents (USA) per kilogram.

Presently, in Turkey, soybean is grown for its oil, cake, and soybean meal used in animal nutrition. In soybean farming areas, oil-crushers have 600,000 tons soybean seed processing capacity in a year (Yaltir, 1980).

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H. Halis Arioglu

2) Research on growing possibilities of some determinate soybean varieties as a second crop in Çukurova, Turkey.

Cukurova region, located in 37°19'N latitude, extends from the Hatay province in the east to the içel province in the west; from the Mediterranean Sea in the south to the Tourus Mountains in the north. It has 1.2 million hectares arable land; l.l million ha of land is used for growing field crops. Cukurova region has the most productive land of Turkey. For this reason, more than 50% of the arable land is irrigated and main crops are cotton and wheat. Because of the suitable climate and soil conditions, it is possible to get more than one crop in a year in Cukurova region (Atakisi and Genç, 1975). In the cotton + wheat growing system, wheat is harvested from the end of May to mid-June. After wheat harvesting, soybean can successfully be grown within 115-135 days in summer time (ölez et al., 1981).

Many INTSOY (International Soybean Program) varieties were taken into research as a second crop and among these varieties 'Amsoy 71', 'Calland', 'Mitchell', 'Mitchell 410', L 4106, A 2575 and A 3127, observed to be suitable for the Çukurova region (Atakisi, 1978; Atakisi and Arioglu, 1980; Arastirma Özetleri, 1986). More than 90% of the total soybean production of Turkey was produced in Çukurova region.

Planting high-yielding varieties besides employing cultural techniques to improve the yield potential of soybean in this region is important. The objective of this study was to find new suitable soybean varieties that can be grown as a second crop in Çukurova region.

Material and methods: This experiment was conducted as a second crop after wheat harvest in experimental field of the University of Çukurova, Faculty of Agriculture, in 1981 and 1982. In this research, sixteen soybean varieties ('Gail', 'Alamo', 'Desoto', 'Bay', 'Davis', 'Williams 79', 'Centennial', 'Celest', 'Imp. Pelican', 'Ufv-l', 'Essex', 'Crawford', 'Ware', 'Braxton', 'Foster', and 'Pk-73-94') which are taken from INTSOY and two local varieties ('Amsoy 71' and 'Calland') were used. The experimental design was randomized block with four replications.

The climatical data over the growing period in Adana in 1981 and 1982 are given in Table 1.

Table 1. The climatic data over growing period in Adana in 1981 and 1982

	Maxi		-Tempe	erature	Aver	200	Relat humidit		To: rainfal	
Months	1981	1982	1981	1982	1981	1982	1981	1982	1981	1982
June	32.5	32.4	19.1	20.2	25.6	25.5	68.2	63.0	125.3	43.1
July	34.0	35.5	23.2	17.1	28.3	27.0	68.9	67.6	-	27.8
August	34.3	37.6	23.6	20.4	28.4	28.0	68.7	69.0	-	69.0
September	35.0	33.9	21.0	21.5	26.8	26.5	59.4	66.5	4.9	15.6
October	31.4	28.3	17.1	15.8	23.0	21.1	58.9	59.4	63.8	79.3

^aSource: Meteorological Surveys of Adana.

The soil type of the experimental area is sandy loam and soil pH ranged between 7.5 and 7.8. The soil contains a high amount of lime. The soil organic matter is low (özbek et al., 1974).

The seedbed was prepared with diskharrow after wheat harvesting. Fertilizer (200 kg ha⁻¹ diammonium phosphate) was broadcast at the time of soil preparation to experimental area. The plot sizes were 2.8 x 5.0 = 14.0 m² and row spacing was 0.7 m. The soybean seeds were inoculated with *Rhizobium japonicum* and planted by hand the first week of June in both years. The seeding rate was 25 plants per m row and planting depth was 5-6 cm. After planting, all plots were irrigated to obtain a uniform emergence of stand. The experimental area was furrowed twice during plant development to control weeds; irrigation was applied by flood irrigation system four times. The plots were harvested by hand at different times when the varieties reached to maturity and threshed by a stationary plot thresher.

INTSOY methods were used to obtain data (Jackobs et al., 1984). The investigated characteristics are:

- Days to maturity (days): Days from date of emergence to date when 95% of the pods were ripe.
- Whitefly scale (1-5): The whitefly observations were made visually according to 1-5 scale (1 = very resistant, 5 = very susceptible) on middle of August in each plot.
- 100-seed weight (g): Weight in grams of 100 randomly selected seeds from dried, cleaned grain.
- Oil and protein content (%): Oil and protein content were determined on the dry weight basis by a near-infrared light reflectance instrument in the Department of Agronomy at the University of Illinois.
- Plant height (cm): Height in centimeters was measured from the ground surface to the top of the main stem at maturity.
- Pod number (pods/plant): Mean number of pods per plant estimated from 29 plants.

The lowest pod height (cm): Height in centimeters was measured from the ground surface to the first pod of main stem.

Yield (kg/ha): Weight in grams in clean, dry grain from 5.0 m of two center rows which is a harvested area of 7.0 m^2 . Yield per hectare estimated from plots yield in kilograms.

Results and discussion: In this study, growing period, whitefly resistance, 100-seed weight, oil and protein percentage, plant height, the lowest pod height, pod number, and seed yield were investigated. Data obtained from the research are summarized in Tables 2 and 3.

Table 2. Growing period, whitefly scale, 100-seed weight, oil percentage, and protein percentage data pertaining to soybean varieties

Varieties	Growing period (days)	Whitefly scale (1-5)	100-seed weight (g)	0i1 (%)	Protein (%)
Gail	147	2	20.10	17.2	45.0
Alamo	175	1	13.04	16.5	43.9
Desoto	121	5	10.42	20.2	43.3
Bay	126	2	19.66	19.6	42.5
Davis	148	3	15.50	18.2	42.4
Williams 79	121	5	14.25	21.1	44.7
Centennial	151	2	14.42	17.2	44.7
Celest	143	5	15.99	17.9	45.4
Imp. Pelican	175	I	13.53	18.1	44.5
Ufv-1	175	1	12.99	18.3	43.5
Essex	126	1	17.16	18.5	44.9
Crawford	133	5	11.72	19.8	44.1
Ware	140	2	20.95	19.0	44.3
Braxton	168	1	17.31	19.6	41.8
Foster	168	I	14.44	18.6	44.1
Pk 73-94	173	1	13.57	17.3	42.5
Amsoy 71	103	I	16.38	19.9	43.0
Calland	110	1	20.49	19.4	44.6

The growing period for soybean varieties was varied between 121 and 175 days. 'Essex' and 'Bay' varieties can be grown as a second crop in the Cukurova region. The other varieties cannot be grown because they have a longer growing period than the required normal time period for the second crop in Cukurova region.

Table 3. Plant height, lowest pod height, pod number, and seed yield data pertaining to soybean varieties

Varieties	Plant rieties height (cm)		Pod number (number/plant)	Yield (kg/ha)	
Gail	99.80 g	21.96 defg	30.99 def	2682.5 ъ	
Alamo	145.78 Ъ	60.93 a	41.39 c	1593.3 f	
Desoto	87.95 h	15.63 hi	29.33 ef	974.6 h	
Bay	111.03 ef	22.78 defg	27.43 fg	3051.9 a	
Davis	118.64 de	21.06 efgh	51.90 a	2576.5 bc	
Williams 79	90.71 h	13.29 i	31.00 def	1060.8 h	
Centennial	112.10 de	22.48 defg	38.74 c	2056.1 d	
Celest	91.03 h	35.76 с	21.38 g	1118.8 gh	
Imp. Pelican	157.34 a	42.30 b	43.71 bc	1157.9 gh	
Ufv-1	161.06 a	41.88 b	49.47 ab	1660.0 ef	
Essex	89.15 h	21.09 efgh	40.16 c	3127.1 a	
Crawford	103.30 fg	22.40 defg	27.81 fg	1405.6 fg	
Ware	75.58 i	17.20 ghi	23.50 fg	1947.9 de	
Braxton	137.03 с	26.46 de	28.65 fg	2139.4 d	
Foster	120.18 d	27.63 d	36.75 cde	1670.8 ef	
Pk 73-94	116.41 de	23.50 def	50.36 ab	2104.8 d	
Amsoy 71	102.35 g	15.95 hi	37.20 cd	2246.7 cd	
Calland	100.86 g	19.44 fgh	30.15 def	2825.8 ab	
EGF (5%)	8.58	5.76	7.62	338.40	
CV (%)	7.71	22.16	21.62	17.35	

Alamo, Imp. Pelican, Ufv-1, Essex, Braxton, Foster, and Pk 73-94 soybean varieties were resistant to whitefly, while Desoto, Williams 79, Celest, and Crawford varieties were very susceptible and the others were moderately resistant.

Mean value of 100-seed weight was, in turn, found to be between $10.42~\mathrm{g}$ and $20.95~\mathrm{g}$ for Desoto and Ware, respectively. The average percentage of oil content of $16~\mathrm{varieties}$ tested varied between 16.5% for Alamo and 20.2% for Desoto. The average percentage of protein content was highest in Celest (45.4%) and lowest in Braxton (41.89%).

The average plant height was determined between $91.03~\rm cm$ and $157.34~\rm cm$ and the lowest pod height ranged between $13.29~\rm cm$ and $60.93~\rm cm$. The lowest pod height was found higher in new varieties than in local varieties.

The average pod number per plant varied between 21.38 and 51.90 in tested soybean varieties. Pod number was highest in Davis and lowest in Celest.

The average seed yield per hectare was found between 974.6 kg and 3127.1 kg. Seed yield was highest in Bay (3051.9 kg/ha^1) and Essex (3127.1 kg/ha^1) soybean varieties. The seed yield of these two varieties was higher than local varieties. A positive correlation was found between the yield, and 100-seed weight and pod number. However, there was a negative correlation between the yield, and oil and protein content, plant height, and lowest pod height. These findings are in agreement with the results of Jackobs et al. (1984).

In conclusion, Bay and Essex soybean varieties can be grown as a second crop after wheat harvest in Cukurova region, Turkey.

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H. Halis Arioglu

3) Screening of some soybean varieties for resistance to whitefly (Bemisia tabaci Genn.).

There are approximately 70,000 hectares soybean planted area in Aegean, Mediterranean, and Southeastern Anatolian regions of Turkey. These regions have the most productive land of Turkey. The main crops in these areas are cotton and wheat. In these regions, soybean is planted as a second crop after wheat harvest. The major insect pest of double-cropped soybean in these regions is sweetpotato whitefly (Bemisia tabaci Genn., Aleyrodidae, Homoptera) which affects soybean and other field crops in Turkey (Çinar et al., 1985). The sweetpotato whitefly has been a very important insect pest since 1974 in Turkey (Şengonca and Yurdakul, 1975).

Whiteflies lay light-yellow, stalked eggs mostly on the underside of leaves. Nymphs are oval and depressed, pale to greenish yellow and 0.5 mm in size. Adults are small insects with yellow body and hialine wings covered with a white powdery wax; it is 1-1.4 mm in size. Injury to soybeans is caused both by nymphs and adults sucking sap from leaves. Whiteflies secrete abundant honeydew. This honeydew forms a suitable medium for the development of a dark sooty mold, which inhibits light penetration and reduces photosynthesis. In addition to injury due to feeding, whiteflies are potentially damaging as vectors of several important soybean viruses (Kogan and Herzog, 1980). Infestation of whiteflies is usually heaviest during the pod-filling period and can cause severe reductions in yield. Chemical control of the whitefly has proven expensive and ineffective (özgür, 1986; özgür and Şekeroglu, 1986). The objective of this research was to identify soybean varieties with resistance to whitefly.

Material and methods: This study was conducted as a second crop after wheat harvest in experimental field of the University of Çukurova, Faculty of Agriculture, in 1976 and 1986. The experimental design was a randomized block with four replications. One hundred and nine soybean varieties were screened in this study, mostly taken from INTSOY and several breeding companies.

The seeds were planted by hand on first week of June. The plot sizes were 2.8 x 5.0 m = $14.0~\text{m}^2$ and row spacing was 0.7 m. The seeds were inoculated with *Rhizobium japonicum* and fertilizer (200 kg/ha⁻¹ diammonium phosphate) was broadcast uniformly over the entire experimental area just prior to soybean planting. Insecticide was not used for whiteflies over the growing period. Since whiteflies were very abundant, no artificial infestation was made. The whitefly observations were made visually according to a 1-5 scale (1 = very resistant, 2 = resistant, 3 = moderately resistant, 4 = susceptible and 5 = very susceptible) in mid-August.

The data for whitefly resistance are presented in Table 1.

Results and discussion: Among the selected varieties, numbers 1-15 were tested in 1976-77, numbers 16-31 in 1978-79, numbers 32-53 in 1981-82, numbers 54-67 in 1982-83, numbers 68-83 in 1983-84, numbers 84-100 in 1984-85 and numbers 101-109 in 1985-86.

Out of 109 soybean varieties tested, 42 varieties were found to be very resistant (1), 25 resistant (2), 16 moderately resistant (3), 14 susceptible (4), and 12 very susceptible (5).

Table 1. The results of screening some soybean varieties for whitefly (1976-1986)

No.	Varieties	Days to maturity	Whitefly scale (1-5)	No.	Varieties	Days to maturity	Whitefly scale (1-5)
1	Amsoy 71	95	1	29	Steele	90	2
2	Beeson	95	1	30	Swift	79	4
3	Chippewa	98	4	31	Altona	73	3
4	Clark	98	5	32	IE	103	2
5	LC-1	98	5	33	Cyst-Co	100	3
6	Sfr-300	98	4	34	Cumberland	98	4
7	Dare	115	3	35	Washington 5	103	1
8	Davis	115	3	36	Victoria	103	3
9	Forrest	115	1	37	Bellati L-263	98	5
10	Mack	115	3	38	Bellati S.D.	98	5
11	Bossier	129	3	39	Shawnee	100	2
12	Bragg	129	4	40	Shawnee 2	98	2
13	Lee 68	129	3	41	Gail	147	2
14	Pickett 71	129	1	42	Alamo	175	1
15	282/2	129	2	43	Desoto	121	5
16	Columbus	113	3	44	Bay	126	2
17	Calland	105	1	45	Williams 79	121	5
18	Cuttler 71	103	2	46	Centennial	151	2
19	Evans	80	3	47	Celest	143	5
20	Franklin	107	3	48	Imp. Pelican	175	1
21	Mitchell	110	1	49	Uvf-1	175	1
22	Corsoy	110	2	50	Ware	140	2
23	Harcor	113	2	51	Braxton	168	1
24	Crawford	113	3	52	Foster	168	1
25	Elf	112	2	53	Pk 73-94	173	1
26	Hodgson	99	2	54	Lakota	89	1
27	Union	90	3	55	Essex	120	1
28	Williams	92	5	56	Hardin	99	2

continued ...

Table 1. Continued

No.	Varieties	Days to maturity	Whitefly scale (1-5)	No.	Varieties	Days to maturity	Whitefly scale (1-5)
57	Hodgson 78	89	2	84	L 4303	95	2
58	Amcord	97	1	85	L 4208	93	3
59	Corsoy 79	95	1	86	L 3665	95	1
60	Clay	89	1	87	L 4204	95	1
61	Kent	105	1	88	L 2330	91	4
62	Century	82	3	89	L 1771	82	4
63	Pixie	82	4	90	L 4404	90	4
64	Williams 82	82	5	91	L 1994	82	4
65	Fayette	80	4	92	L 1808	95	1
66	Pella	80	4	93	L 4209	97	1
67	Sparks	80	4	94	L 4256	83	5
68	Hark	99	1	95	L 4104	93	3
69	Proto	99	1	96	L 4207	95	2
70	Villis	99	2	97	L 4106	115	1
71	Lincoln	102	2	98	L 4206	96	1
72	Adams	104	1	99	Mitchell 450	120	2
73	Vickery	103	1	100	Mitchell 410	110	1
74	Banas	104	1	101	A 1937	88	1
75	Icr	90	2	102	A 2575	95	1
76	Comerto	115	2	103	A 2943	100	1
77	Clark 63	100	5	104	A 3127	105	1
78	Woodworth	90	5	105	Semu-4	103	1
79	2180	99	2	106	Semu-33	103	2
80	2877	102	1	107	Semu-31	100	1
81	3377	104	1	108	Semu-2	99	3
82	3105	103	1	109	Semu-62	96	1
83	1617	103	1				

Whitefly scale: 1 = very resistant

^{2 =} resistant

^{3 =} moderately resistant

^{4 =} susceptible

^{5 =} very susceptible

The resistant varieties (Amsoy 71, Calland, Mitchell, Mitchell 410, Mitchell 450, L 4106, L 4207, Washington 5, Shawnee 2, A 3127, A 2943, A 1937 and A 2575) were recommended for production and are now widely used. Since these varieties had high seed production, they were easily accepted by growers.

No relation was found between whitefly injury and growing period. Woodworth variety was very susceptible and Amsoy 71 was highly resistant, both of which had 90-day growing periods. Similarly, Desoto variety, with 120-day growing period, showed high susceptibility and Essex, with same growing period, showed high resistance. The injured varieties reached seed maturity very quickly and gave low yield, whereas resistant varieties yielded between 2.5 to 4.5 tons per hectare according to cultural practices.

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H. Halis Arioglu

4) Effect of cheese whey as a fertilizer on the increase of soybean nodules.

Cheese whey, a dairy by-product with an estimated world production of 72 million tons, is a polluter to the environment if it is not treated before disposal. Among some practical and simple uses of whey is the use of it as a liquid fertilizer, which was the case in the pastures of USA and many other countries in the 1960s (Ryder, 1980). Since whey contains 50-55% of the dry matter of whole milk, it is rich in organic carbohydrate, fat, and some proteins and inorganic matter (mineral matter) and also in mostly lactic acid bacteria; 3 tons of whey equals 1 ton of animal fertilizer.

The wonder-plant soybean is also becoming popular in Turkey. Soybeans, among their uncountable qualities, have the important quality of enriching the soil with nitrogen. Being a leguminosae, they fix the air N into the soil through the nitrogen-fixing bacteria in their nodules.

In this experiment, cheese whey was used as liquid fertilizer in soybean production. Its effect on increasing the number of nodules was more pronounced and important than on the increase of soybean production.

<u>Materials and methods</u>: In the experiment, 'Calland' variety soybean and cheese whey from the local state dairy factory were used. The composition of the whey was as in Table 1.

Table 1. The composition of cheese whey which was used for the experiment

Drymatter	Protein	Lactose	Fat	Ash	рН	Acidity
6.59%	1.3%	3.99%		0.55%	6.4	6.8 SH

The experiment was carried out in the faculty experimental fields with $12~\text{m}^2$ parcels each, as random block experimental design. Calland variety of soybean seeds treated with the usual microorganism were planted (60 x 5 cm) by hand. Cheese whey was given soon after the planting at 2, 4 and 6 tons/da (1000 m²) rates, while control parcels received only 2-6 tons of water and represented "O ton/da" samples. Soybeans were irrigated three more times during the growth period, but with only water.

Nodules were counted twice, once at the beginning of 50% flowering, and the second time at the beginning of 50% fruit development stages. Nodule counting was carried out according to "INTSOY" (Anon., 1981). Ten plants were chosen randomly from each parcel and used for counting. Active and passive nodules were also determined, again using the methods of INTSOY.

Statistical analyses have been applied to the figures and the results have been summarized in tables and figures.

Results and discussion: The results of soybean nodule counts at two different stages of development and the statistical analysis are given in Tables 2 and 3.

Table 2. Effects of whey as fertilizer on soybean nodules at 50% flowering stage and the statistical results

Whey		Nur	mber of nodu	ıles		Mean
applied		I.block	II.block	III.block	Mean	as %
O ton/da (c 2 tons/da 4 tons/da 6 tons/da	ontrol)	70 178 168 135	68 158 132 139	65 141 210 243	67.7 159. 170.	100 234.9 251.1 254.5
Sources	D.F.	M.S.				
Columns Treatments Error	2 3 6	170.1 7518.3 1307.22	1.3012 5.7514*	-		
Total	11	1307.22		F _{3,6,0.05} = 8	4.76	

As can be seen in Table 2, the numbers of nodules have increased from 67.7 to 159, 170 and 172.3, with 2, 4 and 6 tons of whey per decar. These increases correspond to 234.9%, 251.1% and 254.5%, respectively, and are statistically significant, too.

Table 3. Effects of whey as fertilizer on soybean nodules at 50% fruit development stage and the statistical results

Whey		Nun	mber of nodu	ıles		Mean
applied		I.block	II.block	III.block	Mean	as %
0 ton/da (con 2 tons/da 4 tons/da	ntrol)	178 286 378	186 226 258	159 234 263	174.3 248.7 299.7	100.0 142.7 171.9
6 tons/da		399 - – – – – –	215	263	292.0	167.5
Sources	D.F.	M.S.	F			
Columns	2	9649.0	5.4388			
Treatments	3	9929.86	5.5971*			
Error	6	1774.11				
Total	11			$F_{3,6,0.05} =$	4.76	

As is seen from the figures in Table 3, when the mean value of control groups is accepted as 100%, the increases in 2, 4, and 6 tons of whey-treated samples are 142.7%, 171.9% and 167.5%, respectively. The statistical analysis of these increases also proves to be significant.

The effect of whey as fertilizer on the increase of soybean nodules can be seen as second degree polynomial regression curves in Figure 1. Regression equations and R^2 values are also given in the figure and R^2 values of 0.966 and 0.994 indicates highly significant relationships.

Table 4. Effects of whey as fertilizer on the active-to-passive nodule ratios at the 50% flowering (A) and 50% fruit development (B) stages

Whey applied		o-passive ios (as %)
app 1100	(A)	(B)
O ton/da (control)	70.6	90.3
2 tons/da	81.76	90.5
4 tons/da	77.70	85.8
6 tons/da	76.50	91.2

Figure 1. Effect of whey as fertilizer on the number of nodules at (A)50% flowering

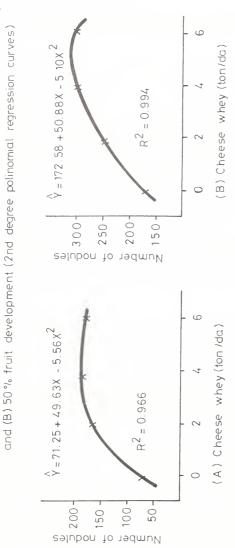


Table 5 summarizes the effect of whey as fertilizer on the levels of soybean production. Applications of 2, 4, and 6 tons of whey per decar increase the soybean yield from 141.7 kg/da to 184.4, 203.4 and 180.5 kg/da, respectively. Though the increases in yields are between 27.4-43.5%, the statistical variance analysis prove them to be insignificant, possibly due to the inconsistent variation figures in yields of treated parcels.

Table 5. Effect of whey as fertilizer on soybean yield

	Soybe	an yields (kg/da)		Mean
Whey applied	I. block	II.block	III.block	Mean	as %
O ton/da (control)	150.	158.3	116.7	141.7	100.
2 tons/da	191.6	211.7	150.0	184.4	130.
4 tons/da	185.	191.7	233.3	203.3	143.5
6 tons/da	183.3	175.0	183.3	180.5	127.

The increase in soybean yield was reasonable since 1 ton of whey as fertilizer would supply 1.5 kg N, 0.4 kg P and 1.5 kg K, as well as some Na, Ca, Mg, and Cl. On the other hand, the statistically significant increase in the nodules seems to be an important observation. Since soybean fixes air N into the soil by its "rhizobium" bacteria in the nodules, this observation of effect of whey as fertilizer on the increase of soybean nodules looks very encouraging and promising as a way to speed up or increase the enrichment of soil with N.

The mechanism of this increase in the number of nodules with whey as fertilizer is not quite clear. Maybe it has something to do with the rich lactic acid bacteria content and with fertilizer value of whey or the high biological oxygen demand (BOD) of whey which fits well with the "nif genes" being fond of less oxygen in the immediate vicinity (Tudge, 1983). Whatever the reason or mechanism for this increase in the nodules of soybean with whey as fertilizer, it seems to be well worth looking into.

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1) Assessment of the behavior of perennial Glycine genotypes in tissue culture.

Perennial Glycine species exhibit a number of characteristics of agronomic potential including daylength neutrality, tolerance to heat, drought, cold (Marshall and Broué, 1981) and salinity (Newell and Hymowitz, 1982), and resistance to soybean cyst nematode (Riggs and Hamblen, 1962, 1966), yellow mosaic virus (Singh et al., 1974), powdery mildew (Mignucci and Chamberlain, 1978) and rust (Burdon and Marshall, 1981). Each of these features would be useful if introgressed into the soybean genepool. However, to date, it has only been possible to produce a few sterile hybrids using conventional crossing followed by embryo rescue (see Newell et al., 1987).

Somatic hybridization by protoplast fusion offers an additional approach for hybrid plant production. An essential aspect of this technology is an ability to regenerate whole plants from isolated protoplasts. Other genetic manipulations using somatic cell techniques, including transformation by isolated DNA or Agrobacterium—mediated delivery of vectors, also demand a knowledge of the factors controlling plant regeneration.

In the past five years, work on *Glycine* in the Plant Genetic Manipulation Group at Nottingham has centered upon the development of techniques for rapidly assessing the response in culture of a range of genotypes of several perennial species. Once received into our seed collection from such sources as AVRDC, CSIRO, or USDA, each accession is monitored for its ability to undergo plant regeneration from explants or explant-derived callus. Subsequently, promising accessions are screened for protoplast release, plant regeneration from protoplasts, and their suitability as a fusion partner with protoplasts of soybean. To date, we have screened at least 30 accessions of six *Glycine* species, including *G. canescens, G. clandestina, G. falcata, G. latifolia, G. latrobeana, G. tabacina*, and *G. tomentella*. This report summarizes the methods used, and our main observations; more detailed information can be obtained from the publications cited.

<u>Materials and methods:</u> <u>Seedling growth.</u> Seeds were surface-sterilized (20 min) in a 10% (v/v) "Domestos" bleach solution, followed by thorough rinsing in sterile tap water. The testas were scarified using a scalpel, and the seeds soaked in sterile tap water for 48 h with a change after 24 h. Seeds were sown on agar-solidified (0.8% w/v) hormone-free Murashige and Skoog (1962) based medium, and germinated in a culture room (continuous fluorescent illumination; 1.6 Wm $^{-2}$; 27°C).

<u>Callus initiation</u>. Ten to 14-day-old seedlings were dissected, and the explants (leaves, petioles, cotyledons, cotyledonary and epicotyl nodes) placed on SC2 agar medium containing B5 salts and vitamins (Gamborg et al., 1968), 1.1 mg/l benzylamino purine (BAP) and 0.005 mg/l indolebutyric acid (IBA) (Hammatt et al., 1986).

Shoot elongation and rooting. Shoot elongation in all species except G. latrobeana was stimulated by using SC6 medium, similar to SC2, but with BAP reduced to 0.2~mg/1. Rooting in all species, and shoot elongation in G. latrobeana, was induced on half-strength, hormone-free B5 medium (HB50).

Isolation and culture of seedling cotyledon protoplasts. Cotyledons were cut transversely and incubated in a solution of 1% Rhozyme HP150, 0.2% Cellulase R10 and 0.1% Pectolyase Y23 dissolved in CPW9M which contained CPW salts (Frearson et al., 1973) and 9% mannitol (Lu et al., 1983). Released protoplasts were filtered through a 45 μm sieve, cleaned by centrifugation through CPW9M solution and plated in agarose beads of Kao (1977) protoplast medium (Hammatt et al., 1987a). Protoplast-derived callus was transferred to SC2 and SC6 media for plant regeneration.

Introduction of cell suspensions and isolation of protoplasts. Hypocotyl and petiole explants were placed on agar-solidified (0.9% w/v) UM medium (Uchimiya and Murashige, 1974), and callus transferred to UM liquid medium in 250 ml Erlenmeyer flasks on a rotary shaker (80 rpm). Log phase cells were incubated in a solution containing 0.4% Driselase, 0.8% Cellulase R10, 0.4% Pectinase, 0.8% Rhozyme HP 150, 0.059% 2-(N-morpholino)ethane sulfonic acid, 0.1% CaCl2·2H2O, 0.01% NaH2PO4·H2O, 6.8% sorbitol and 6.8% mannitol. Protoplast culture conditions were similar to those used for protoplasts from seedling cotyledons.

Results and discussion: Cotyledonary and epicotyl nodes from all species except *G. latifolia* and *G. tabacina* underwent extensive lateral branching on SC2 medium. This response was similar to that described previously for soybean (Barwale et al., 1986). The resulting bud/shoot cultures could be subcultured indefinitely to produce clonal material suitable for further *in vitro* studies, and eliminating the requirement for large numbers of seeds. This was particularly useful for *G. falcata* and *G. latrobeana* in which seed production is poor under UK greenhouse conditions.

Although there were differences in the response of species, accessions and explants to SC2 medium, seedling cotyledons, leaves and petioles of *G. canescens, G. clandestina, G. falcata, G. latrobeana*, and *G. tomentella* produced callus from which shoot buds developed. Such buds formed shoots following transfer of tissues to SC6 medium, and the shoots rooted on HB50 medium. In the case of *G. latrobeana*, the intermediate step on SC6 medium was unnecessary, since shoot extension and rooting both occurred on HB50 medium. *G. latifolia* and *G. tabacina* formed callus, but the latter failed to produce buds on SC2 medium. Plant regeneration was most efficient in *G. canescens* G1171 and *G. clandestina* G1231 (Hammatt et al., 1986, 1987b).

Seedling cotyledons of all species evaluated released protoplasts, which, with the exception of *G. latrobeana*, could be cultured to callus. Although it does not always follow that accessions capable of plant production from explants will also regenerate from protoplast-derived callus, it was possible in the case of *G. canescens* (G1171) and *G. clandestina* (G1231) to recover plants readily from tissues of protoplast origin (Hammatt et al., 1987a)

Suspension cultures of perennial *Glycine* species have provided a continuous supply of cells for the isolation of protoplasts, and the latter have been cultured to callus. Currently, plant regeneration studies with this material are still in progress. Suspension cultures should prove useful as a

source of large numbers of protoplasts in accessions where seed supplies are limited, since only a single seedling is required as a source of hypocotyl and petiole explants for culture initiation.

Recent advances in the identification of perennial *Glycine* accessions capable of plant regeneration from explant and protoplast-derived callus now permit a number of wild species to be incorporated into programs employing somatic cell techniques to transfer agronomically useful traits into the cultivated crop. This knowledge, combined with the ability to select large populations of fused protoplasts by automated flow cytometry (Afonso et al., 1985), should enable the production of hybrid cells and novel plants to be a realistic possibility in the immediate future.

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1) Measured and predicted yield response of soybeans to simulated acid rain.

Modern soybean cultivars in the U.S. are highly related within the northern and within the southern maturity groups and they trace their genetic constitution to a small number of ancestral lines. On the other hand, there is much less genetic relationship between northern cultivars and southern cultivars. Examples of this can be seen in Figure 1, which shows the ancestral relationships among selected lines and cultivars developed from the northern and southern germplasm pools, respectively. Slightly more than 80% of the northern cultivars released during 1971-81 trace their genetic makeup to 10 accessions (Delannay et al., 1983). 'Mandarin', alone, accounts for more than 30% of the genes in the northern cultivar gene pool. Similarly, more than 80% of the genes in southern cultivars released during 1971-81 come from seven accessions. According to Delannay et al. (1983), CNS and S100 have contributed more than 50% of the genes in the southern cultivar gene pool. This narrow genetic base is a major concern to the breeders and geneticists due to vulnerability to diseases, insects, etc. On the other hand, the narrowness of the genetic base facilitates the assessment of future progenies! reactions to environmental changes. We are attempting to predict the response of soybean lines to acid rain on the basis of the reaction of the ancestral lines to acid rain.

Current knowledge concerning soybean response to acid precipitation is inconclusive in that stimulation, inhibition, or no effect on yield has been reported (Evans and Thompson, 1984; Evans et al., 1981; Heagle et al., 1983; Irving and Miller, 1981; Troiano et al., 1983). This uncertainty in response might be due to the differing growing conditions. Soybeans grown under optimum conditions might tend to overcome adverse effects of acid precipitation whereas stressful growing conditions might adversely affect productivity.

The objectives of our research were to evaluate: (1) yield response of ancestral lines and cultivars derived from them to Simulated Acid Rain (SAR); (2) interaction between environments and SAR for yield; and (3) predicted yield response to SAR of progeny from matings among ancestral lines.

Materials and methods: During 1985, the experiments were conducted at Knoxville and Milan, Tennessee. At each location, the 72 treatment combinations (optimum vs. sub-optimum soil fertility, three levels of SAR acidity, and 12 entries) were grown in a split-split strip block arrangement with three replications. The two soil fertility levels served as main strips, the three SAR treatments (pH 2.8, pH 3.2, and pH 4.3) served as the split-block and 12 entries served as the split-split block. The results of soil test were as follows: water pH of 5.9; buffer pH of 7.7; 46 kg/h P205; and 80 kg/h K20. In order to create two types of growing environments, lime (5.6 mt/h ground limestone) and fertilizer (89.6 kg/h P205 and 179.3 kg/h K20) were incorporated into the optimum strips to raise their fertility and soil pH to optimum levels. The sub-optimum strips did not receive any lime or fertilizer. Each experimental unit consisted of three rows 3 m in length with 0.9 m space between the rows.

The entries included eight ancestral lines ('AK Harrow', 'CNS', 'Manchu', 'Mandarin', PI 54610, 'Richland', S 100, and 'Tokyo') and four cultivars ('Amsoy 71', 'Essex', 'Lee 74' and 'Williams 82'). The SAR treatments were applied (spray-until-runoff) three times a week at Knoxville and two times a week at Milan from V2 until R7 stage. The amount of SAR represents mean weekly amounts of ambient rain based on the 30-year mean. The SAR solutions were made using the composition of rainfall as reported by Cogbill and Likens, a l:l mix of nitric and sulphuric acids substantiated with ammonium sulfate, calcium sulfate, sodium sulfate, potassium sulfate, and magnesium sulfate. The SAR was applied with a tractor-mounted sprayer at a pressure of 0.7 kg/sq cm using a PTO-driven diaphragm pump. The SAR quality was maintained by analysis of time of application samples for pH and other parameters.

The entries were hand-harvested and threshed in a plot thresher and their yields were recorded. The data were analyzed using SAS procedures (SAS Institute, Inc., Box 8000, Cary, North Carolina 27511-8000). The yields of four cultivars (Amsoy 71, Essex, Lee 74, and Williams 82) were predicted from ancestral lines' yield response and the covariance relationships among ancestral lines and these cultivars by using mixed models procedure (Henderson, 1975; Hill and Rosenberger, 1985). The covariance matrix for relationship among ancestral lines and cultivars was generated by using 'PROC INBREED' procedure in SAS. The ratio of phenotypic variance to genetic variance (the heritability of yield in soybeans assumed to be .4) was superimposed on the covariance matrix. We used a generalized inverse of covariance matrix to

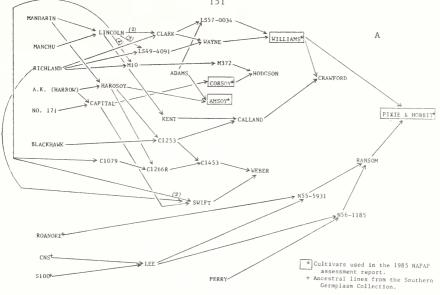
obtain Best Linear Unbiased Prediction (BLUP) for yield response of cultivars to different environments. The yield data of these cultivars was eliminated, one cultivar at a time, before obtaining the BLUP estimate. The BLUP estimates of yield were compared to the actual yield of these cultivars.

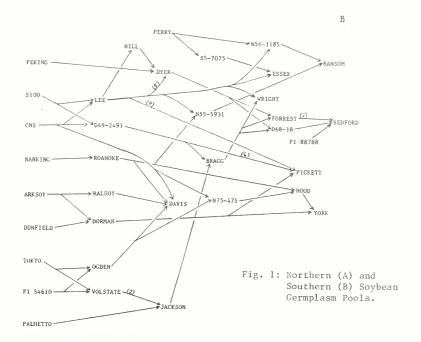
Results and discussion: There was significant interaction between locations, entries, soil fertility levels, and acidity levels of SAR. At both locations, there was significant interaction between entries, soil fertility levels, and acidity levels of SAR except between entries*soil fertility levels and soil fertility levels*acidity levels of SAR at Knoxville.

At Knoxville, an increase in the acidity of SAR from pH 4.3 to pH 2.8 significantly decreased the yield of Manchu (28.8%) at optimum soil fertility and significantly increased the yields of Mandarin (51.4%) and Tokyo (36.5%) at sub-optimum soil fertility (Table 1). At Milan, an increase in the acidity of SAR from pH 4.3 to pH 3.2 significantly increased the yield of AK Harrow (124.7%) and significantly decreased the yield of Amsoy 71 (9.8%) under optimum soil fertility and any further increase in the acidity of SAR did not have any significant effect. The increase in the acidity of SAR from pH 4.3 to pH 2.8 significantly increased the yields of Richland (171.3%) and Tokyo (22.5%) at optimum soil fertility.

The predicted and actual yields of four cultivars are presented in Table 2. The predicted yields of Essex, Lee 74, and Williams 82 were always lower than their actual yields in both locations. The predicted yields of Amsoy 71 were always higher than its actual yields except at pH 3.2 with optimum soil fertility at Knoxville. At Milan, the predicted yields of Amsoy 71 were either greater than or similar to its actual yields. Based on their genetic background, Essex, Lee 74, and Williams 82 were expected to yield lower than their actual yields, indicating that they were not detrimentally affected by the acid rain. On the other hand, Amsoy 71 did not yield as expected from its genetic background, indicating that it might be sensitive to acid rain.

The comparison of actual yields (Table 1) did not give conclusive results but still gives an indication that Amsoy 71 is sensitive to acid rain under some growing conditions. The parents of this cultivar (Figure 1) are Harosoy and Adams. The parents of Harosoy, AK Harrow and Mandarin, were not detrimentally affected by SAR (Table 1). Therefore, the sensitivity of Amsoy 71 apparently was not inherited from either AK Harrow or Mandarin. Since Adams





Mean yield (g/plot) of eight ancestral soybean lines and four cultivars with three pH levels simulated acid rain Table 1.

			- Knoxville	.11e —					Milan	n		Y
	-qnS	Sub-optimum			Optimum		Sub	Sub-optimum		0	Optimum	
Entry	2.8	3.2	4.3	2.8	3.2	4.3	2.8	3.2	4.3	2.8	3.2	4.3
AK Harrow	392a*	388a	391a	446a	417a	441a	253a	266a	222a	241ab	346a	154b
Amsoy 71 [§]	442a	428a	372a	593a	582a	575a	364a	370a	374a	392ab	378b	419a
CNS	399а	440a	455a	500a	398a	709a	288a	303a	291a	340a	338a	389a
Essex	549a	735a	683a	831a	795a	821a	806a	619a	709a	831a	804a	659a
Lee 74 [§]	524a	568a	556a	694a	617a	561a	476a	586a	521a	635a	718a	679a
Manchu	474a	378a	409a	430b	468ab	604a	337a	276a	268a	368a	304a	2773
Mandarin	480a	366b	317b	526a	402a	426a	294a	291a	281a	288a	278a	224a
PI 54610	473a	421a	546a	689a	739a	667a	364a	374a	484a	461a	529a	459a
Richland	377a	366a	357a	512a	429a	467a	278a	230a	225a	293a	209ab	108b
8100	1		1	1	1	1	438a	467a	390a	442a	449a	383a
Tokyo	864a	533b	633b	851a	588a	1043a	429a	428a	467a	604a	545ab	493b
Williams 82 [§]	536a	567a	609a	813a	819a	730a	580a	559a	588a	710a	643a	597a

[†]Levels of soil fertility.

†pH of simulated acid rain.

§Cultivar.

entry (for example, in case of AK Harrow, the yields of three pH levels under sub-optimum fertility in Knoxville can only be compared with each other). Means followed by same letters are not different according to * Comparison of means to be made within locations, within each fertility level and separately for each Duncan's Multiple Range Test (P = 0.05).

 ${\rm Actual}^{\dagger}$ and ${\rm predicted}^{\ddagger}$ yields (g/plot) of four soybean cultivars with three pH levels of simulated acid rain Table 2.

				Д	pH Levels of		simulated acid rain	id rain				
	-	— рн 2.8	8.3			— рн 3.2	.2			— рн 4.3	.3	
	Ŋ.	KES *	MES#	# 5	KES		MES		KES		MES	
Entry	Sub	Opt¶	Sub	Opt	Sub	Opt	Sub	Opt	Sub	Opt	Sub	Opt
Amsoy 71	442 [†] (493) [‡]	593 (608)	364 (374)	392 (415)	428 (456)	582 (543)	370 (363)	378 (413)	372 (457)	575 (587)	374 (365)	419 (337)
Essex	549 (442)	831 (624)	806	831 (472)	735 (495)	795 (558)	619 (425)	804 (495)	(683 (499)	821 (621)	709 (400)	659 (455)
Lee 74	524 (474)	(679) 769	476 (476)	635 (507)	568 (552)	617 (594)	586 (440)	718 (500)	556 (537)	561 (712)	521 (437)	679 (448)
Williams 82	536 (468)	813 (552)	580 (344)	710 (376)	567 (423)	819 (502)	559	643	609 (418)	730 (579)	588	597 (309)

* Knoxville Experiment Station.

 $^{\#}$ Milan Experiment Station.

 $^\S{\rm Sub-optimum}$ soil fertility and pH.

 $^{\rm ff}{\rm Optimum}$ soil fertility and pH.

was not included in this study, no definite statement can be made about its contribution to the sensitivity of Amsoy 71 but it remains a suspect. Adams and Manchu (another sensitive line) were involved in the pedigree of Williams 82, which was not detrimentally affected by SAR. It is possible that the contributions made by Mandarin and Richland to the pedigree of Williams 82 outweighed the sensitivity contributed by Manchu and/or Adams. Evans and Thompson (1984) also observed a decrease in the seed yield of Amsoy 71 with an increase in acidity of simulated acid rain. The actual yields of Amsoy 71 under optimum soil fertility conditions at Milan decreased with increasing acidity of SAR. The actual yields of Essex, Lee 74, and Williams 82 either increased with increasing acidity of SAR or were similar at all acidity levels.

The use of mixed models to predict the yields of future progeny seems quite worthwhile. It might be used successfully to predict the performance of future progeny under varying environmental conditions. This technique was compared with six other techniques by Hill and Rosenberger (1985) for estimating yields of lines included in a series of trials that did not contain all entries in equal numbers. They concluded that Best Linear Unbiased Prediction (BLUP) method was most desirable since it gave smallest prediction errors.

Besides yield, data on maturity and plant height were also recorded on all plots at both the locations. In addition, transpiration rate and stomatal resistance were recorded for AK Harrow, CNS, Essex, Mandarin, and Williams 82 at Knoxville. The effects of SAR on plant height and maturity were not significant. The transpiration rate of Essex decreased, whereas that of CNS increased with increasing pH of SAR.

The results from one year at two locations indicate that (1) Essex, Lee 74, and Williams 82 were not detrimentally affected by the acid rain; (2) Amsoy 71 seems to be sensitive to SAR at least under some growing conditions; (3) the growing environment seems to affect the response of soybean cultivars and lines to acid rain; and (4) the comparison of actual and predicted yields might be a useful criterion in assessing sensitivities of soybean lines and cultivars to acid rain.

Additional greenhouse (at Knoxville) and field experiments (at Knoxville and Milan, Tennessee) will be conducted during 1986 to further assess the impact of acid rain on soybean growth and yield.

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1) Seed production by male-sterile soybeans in Missouri.

The production of hybrid soybean will require the discovery of \mathbf{F}_1 heterosis of a magnitude that provides growers the incentive to purchase hybrid seed. The primary barrier to the testing of \mathbf{F}_1 heterosis is the acquisition of adequate quantities of \mathbf{F}_1 seed (Nelson and Bernard, 1984). Presently, the most efficient means of obtaining experimental quantities of hybrid seed is through the use of nuclear (genic) male-sterile mutations. We have been producing hybrids via male sterility, and in the process have recorded observations on seed production by male-sterile plants. Among the male-sterile lines available in Maturity Groups III and IV, we have detected differences in the efficiency of seed production. Results obtained during the growing seasons of 1984 and 1985 may be of interest to those using male-sterile lines for hybrid formation or in random mating populations.

Lines used carried one of the following male-sterile mutations: ms1-Urbana (Ums1), ms1-North Carolina (NCms1), ms2 or ms3 (represented by Soybean Genetic Type Collection numbers T266H, T260H, T259H and T273H, respectively). Three of the lines used were developed from the original genetic stocks and are maintained as pure lines segregating for male sterility. In addition, two near-isogenic lines, one with ms2 (Williamsms2) and one with NCms1 (Clarkms1) were used. Experiments were conducted at the Monsanto Research Farm, St. Charles, Missouri, in 1984 and 1985. Lines segregating for male sterility were planted in a series of isolation blocks (15 in 1984, 13 in 1985). Each block contained a unique pollen parent, plus either three (1984) or five (1985) male-sterile lines. The experiment occupied an area of 2 ha. In 1984, four hives of approximately 10,000 honey bees were placed in the field. In 1985, eight hives were used. In addition, 10,000 alfalfa leafcutter bees were added in 1985. At flowering, fertile segregants were rogued from the male-sterile populations. Plants were harvested individually at maturity, and the number of seed per plant determined.

For statistical analysis, comparisons were made within years only. Using individual plants as replications, mean seed set was determined for each combination. Weighted means were calculated for each male-sterile parent (averaged over pollen parents) and for each pollen parent (averaged over male-sterile parent). Differences then were determined through use of the

Tukey-Kramer method for comparing means based on unequal sample sizes (Sokal and Rohlf, 1981). Mean seed set was not analyzed by combination.

Mean seed sets for the years 1984 and 1985 are given in Tables 1 and 2, respectively. In 1984, seed production was quite low, and no differences were detected among male-sterile lines. In 1985, seed yield increased dramatically, and differences between male-sterile lines were obvious. The higher seed production in 1985 may be a result of the increased bee density. However, the month of July, 1984 (peak flowering period), was characterized by widely fluctuating temperatures, with 12 days having a maximum temperature of less than 28°C. Low daytime temperatures have been shown to negatively influence attributes of soybean flowers responsible for insect attractiveness (Robacker et al., 1983). Thus, the combination of fewer insects and less favorable climatic factors may have been responsible for the low yields in 1984. Within a given year, all pollen parents were equally effective as pollen donors; no differences were detected in mean seed set as a function of the male parent.

Our results suggest that differences exist among the available malesterile lines in regards to the production of hybrid seed, providing climatic factors and availability of insects are not limiting. The observed differences in 1985 among the male-sterile lines may be the result of differences in floral characteristics responsible for outcrossing. According to Erickson (1975), such differences exist among soybean cultivars. Differential pleiotropic effects of the various male-sterile mutations on female fertility also may be involved. Of the male-sterile lines available in Maturity Groups III and IV, lines carrying ms2 are most prolific in seed production. This study, along with that of Carter et al. (1983), demonstrates that mean seed yields in excess of 100 per plant can be achieved with lines carrying ms2. Breeders interested in generating F_1 hybrid seed, or in developing new populations or isolines carrying male sterility, would be most successful if ms2 were employed.

Table 1. Seed production by male-sterile plants, 1984. N = number of plants. Means followed by the same letter did not differ significantly at the 0.05 level of probability

	M	ale-sterile paren	t	Weighed means
Pollen parent	Т266Н	Williams ms2	Т273Н	Total N
Boone mean	8.7 7	6.7 3	9.0 2	8.1 a 12
Delmar	5.8 10	8.8 5	7.8 5	7.5 a 20
Elf	8.0 7	7.6 8	2.5	6.0 a 17
Mead	6.9 10	14.2	5.0 3	8.7 a 19
Wayne	44.0	28.3	22.7	31.7 a 9
PI 65379	46.0	9.3 3	11.3	22.2 a 9
PI 84680	4.0 5	7.0 2	3.7 3	4.9 a 10
PI 85469	8.7 6	7.0 2	9.6 5	8.4 a 13
PI 86136	22.5	20.0	7.0 2	16.5 a 12
PI 88459	14.3 10	18.3 4	12.3	15.0 a 18
PI 89010	9.3 7	24.0 3	11.5 4	14.9 a 14
PI 89061-2	12.8 7	7.0 5	5.0 2	8.3 a 14
PI 91089	11.9	12.2 14	7.5 2	10.5 a 25
PI 91142	14.7	12.5 6	17.0 5	14.7 a 17
PI 229738	37.7 6	18.3 7	11.0	22.3 a 16
Weighted means Total N	17.0 a 104	13.4 a 73	13.3 a 48	

Table 2. Seed set on male-sterile plants, 1985

Pollen parent		Ma1	e-sterile pare	ent		Weighted means
rollen parent	Т266Н	Clark ms1	Williams <i>ms1</i>	Т259Н	Т273Н	Total N
E1f	39.7	5.6	115.0	85.8	52.4	59.7 a
	11	8	19	18	9	65
Union	68.3 3	12.5	163.3 12	109.5 4	49.8 12	80.7 a 33
Wayne	43.0	6.2	126.1	116.2	64.2	71.1 a
	9	10	14	17	14	64
T273H-F*	33.4 21	13.0	76.0 2	101.2 11	42.8 15	53.3 a 57
PI 65379	65.6 9	16.8	180.5 15	118.7 9	41.2 13	84.6 a 50
PI 70212	30.9	6.0	131.8	61.6	43.9	54.8 a
	7	2	10	9	16	44
PI 84656	34.8	8.2	85.5	79.6	32.5	48.1 a
	15	5	20	15	16	71
PI 86136	56.1	12.0	68.9	51.0	41.6	45.9 a
	9	9	21	6	14	59
PI 88456	31.2 12	8.0	88.3 10	24.2 11	24.4 18	35.2 a 53
PI 89061-2	44.5	9.9	98.1	48.5	21.2	44.4 a
	13	10	14	13	23	73
PI 91750	45.2	9.0	199.0	98.1	36.9	77.6 a
	11	6	6	16	18	57
PI 181554	80.0	12.0	141.6	54.5	45.9	66.9 a
	7	20	14	2	15	58
PI 229738	52.0	8.4 9	171.8	112.4	57.4 12	80.7 33
Wtd. means	48.1 b	c 9.8 bc	126.6 a	81.6 ab	42.6 b	С
Total N	129	95	162	136	195	

^{*}Fertile (nonsegregating) isoline of T273H.

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1) A ploidy variant regenerated from embryogenic tissue cultures of soybean.

<u>Introduction</u>: A potential application of plant cell and tissue culture to plant improvement is likely to be the use of variation generated by passage through cell and tissue culture (somaclonal variation). With the advent of regeneration protocols for soybean (Christianson et al., 1983; Lazzeri et al., 1985; Ranch et al., 1986; Barwale et al., 1986; Wright et al., 1986), it becomes necessary to document the occurrence and frequency of variant phenotypes in progeny of regenerated plants. We report here preliminary findings on the identification of a chromosomal variant in plants regenerated from embryogenic tissue cultures of soybean.

Materials and methods: Immature embryos of soybean were prepared as described (Ranch et al., 1985, 1986). Briefly, immature seed were dissected from pods of field-grown plants and sterilized with 5% Clorox for 15 min. Immature embryos 4- to 6-mm long were removed from the immature seed and cultured with the abaxial surface of one cotyledon laid on semisolid MS medium (Murashige and Skoog, 1962) + 20 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) + 0.7% agar. The cultures were maintained under 100-lux continuous illumination from fluorescent lamps. After two to six weeks, somatic embryos organized upon the margins of the cotyledons. Immature somatic embryos were colorless to pale yellow structures, 0.5 mm to 2 mm in length, resembling most closely a globular stage zygotic embryo. These embryos were removed from the cotyledon and transferred to semisolid MS + 10% sucrose + 0.5% activated charcoal without growth regulators. After two weeks, the immature embryos possessed well-formed radicles and cotyledons. These matured somatic embryos were transferred to semisolid MS + 3% sucrose without growth regulators. Embryos were transferred to fresh medium at 10- to 14-day intervals. The embryos germinated within 6 weeks. Germinated embryos were maintained on semisolid MS in 125 x 25-mm culture tubes for plantlet propagation. After the plantlets produced 3 to 5 nodes, the regenerated plants were potted in soil and hardened in a greenhouse. Seed was collected after self-fertilization.

Analysis of progeny from regenerated plants: Regenerated soybean plants were initially generated as a by-product during the development of a protocol for plant regeneration from embryogenic tissue cultures. There was no experimental design to quantitate any material derived from these initial regenerants. Because regenerated plants often possess developmental abnormalities that are not sexually transmitted, the regenerated soybean plants (R_1 generation) were not observed for specific traits. Regenerated plants were permitted to self-pollinate. If any variants were to appear in subsequent sexual generations, it is likely that the variant phenotype would be genetic in nature.

All seed from regenerated plants (R_2) were planted at 10 seed/foot in 8-foot rows. The initial planting of progeny from regenerated plants was in

1985 near Champaign, IL. In 1986, additional R_2 , and R_3 seed harvested from R_2 plants in 1985 were planted with maturity checks and sexually-derived control plantings from seed of the genotypes regenerated. Seed from a single regenerated plant, or progeny thereof, were bulked at each generation for subsequent year's testing.

Twenty-seven percent of the families possessed visible variation (Table 1). The R2 and R3 generations described here were derived from distinct and different regenerated plants. The R3 families were not evaluated as the R2 in 1985. There were apparent alterations in fertility, maturity, height and variegation. No variants in pubescence or flower color were observed. All variants will be subjected to further genetic study for characterization. Here, we would like to report on a case of ploidy variation apparently induced by passage through tissue culture. In some cases, there was apparent segregation within a family for a variant phenotype, while in other families, the entire population exhibited the altered trait.

Table 1. Tabulation of variant families detected in selfed sexual generations following plant regeneration from embryogenic tissue cultures in soybeans

R ₂ total families	R2 variant families	R3 total families	R ₃ variant families
21	5	2	2
15	2	5	3
2	1	-	-
6	1	-	-
	families 21 15 2	families families 21 5 15 2 2 1	families families families 21 5 2 15 2 5 2 1 -

A single R₂ family of 'Gem' (Group II) occupying 1 row was observed to be morphologically abnormal. Four plants were present in the row, but it is unknown how many seed were planted. These four plants possessed large thick leaves, low and variable seed set, and remained green until harvest. The pollen from one of these plants was observed to possess 4 to 5 colpi, whereas normal pollen has 3 colpi. Chromosome analysis (Palmer and Heer, 1973) on R₃ seed derived from these plants revealed that the plants possessed approximately 80 chromosomes (Table 2).

Table 2. Chromosome number of ${\bf R}_3$ progeny originating from the four ${\bf R}_1$ plants

Plant no.	No. seed	Chromosome no.
75-1	16	ca. 80
75-2	7	ca. 80
75-3	11	ca. 80
75-4	35	ca. 80

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1) New mutations in a genetically unstable line of soybean.

Most plants of the Asgrow Mutable line of soybean are chimeric for flower color (Groose and Palmer, 1986). Mutable plants produce both entirely near-white and entirely purple flowers, as well as flowers of mutable phenotype with purple sectors on near-white petals. This line carries an unstable recessive ('mutable') allele of the w4 locus that conditions anthocyanin pigmentation (Weigelt et al., 1986). The mutable allele reverts at high frequency from the recessive state to a stable dominant state. Many such mutable alleles in plants have been analyzed at the molecular level and in every instance the action of a transposable element has been established (Doring and Starlinger, 1986). We hypothesize that the Asgrow Mutable line harbors an active transposable element system and that the high frequency of reversion of the unstable allele results from excision of the putative element from the w4 locus.

The objective of our study was to recover new mutations at other loci in the Asgrow Mutable line as evidence for transposition of a mobile genetic element. We reasoned that the probability of recovering new mutations might be maximized by searching for mutants among progenies of wildtype (germinal revertant) progeny of mutable plants. A germinal revertant is the result of a reversion of the unstable allele in the germline of a mutable parent. Germinal revertants produce only wildtype purple flowers and their self progenies either breed true for wildtype pigmentation or segregate 3 wildtype:1 mutable. If a reversion of the unstable allele is the result of transposition of the positive element out of the w4 locus and into another locus, then new mutations at other loci might be detected among the progenies of germinal revertants. Our strategy was to survey progenies of many germinal revertants (each of which was derived from an independent reversion event) for new mutations at as many loci as possible.

A summary of the study is presented in this communication. Three accompanying research notes describe new mutations for chlorophyll deficiency (Groose et al., 1987), partial sterility (Groose and Palmer, 1987) and necrotic roots (Blomgren et al., 1987).

<u>Materials and methods</u>: The experiment was conducted as follows: $\underbrace{\text{Step 1}}_{\text{: }} \text{ (F}_{9} \text{ generation; Field nursery, Ames, Iowa, 1985)}. \text{ Two thousand } \\ \text{mutable plants were selected from F}_{9} \text{ progeny rows that descended from 60} \\ \text{highly mutable F}_{8} \text{ plants of the Asgrow Mutable line. Each F}_{9} \text{ plant was threshed separately to produce 2000 F}_{10} \text{ families.}$

 $\underline{\text{Step 2}}\text{:}$ (F $_{10}$ generation; Off-season nursery, Puerto Rico, winter 1985-86). Approximately 30 seed of each of the 2000 F_{10} families were planted to produce an $F_{10}\text{-progeny}$ row. A single germinal revertant was selected from each row that contained at least one germinal revertant (1599 rows). Selection of a single germinal revertant from each progeny row assured that every germinal revertant was derived from an independent reversion event. For each row that produced no germinal revertants (401 rows), a single mutable plant was selected. Selected plants were threshed separately to produce 2000 F_{11} families.

Step 3: (Fl1 generation; Greenhouse sandbench and field nursery, Ames, Iowa, 1986). Sufficient seed was available to test 1936 and 1697 Fl1 families, respectively, in a greenhouse sandbench and in a field nursery. In the sandbench, approximately 25 seedlings in each family were observed for segregation for new mutations until the second trifoliolate leaf stage when plants were pulled from the bench for examination of root systems. In the field nursery, approximately 25 plants in each family were observed periodically throughout the season and at maturity for segregation for new mutations. In both locations, progenies were surveyed for characters that are easily evaluated by visual examination. These included chlorophyll pigmentation, root fluorescence, seed pigmentation, leaf form, sterility, dwarfness, stem and petiole morphology, and time of flowering and maturity. Dominant alleles of more than 50 described nuclear loci condition the wildtype phenotypes of these traits (Palmer and Kilen, 1987) and the Asgrow Mutable line breeds true for wildtype for all these traits.

Most new mutations that result from insertion of a transposable element are expected to be recessive. In this experiment, some F_{10} plants are expected to descend from F_9 -germline sectors that carry new recessive mutations. Mutant F_9 -germline sectors are expected to be heterozygous for the new mutations and to produce F_{10} progeny that segregate 1 homozygous wildtype:2 heterozygous:1 homozygous recessive. Therefore, 25%, 50%, and 25%, respectively, of F_{11} families that descend from F_9 -germline sectors with new nonlethal recessive mutations are expected to breed true for wildtype, segregate 3 wild-type:1 recessive, and breed true for the recessive phenotype. Deleterious mutations are expected to eliminate some homozygous recessive plants, alter segregation ratios, and reduce the probability of recovering F_{11} families that breed true for recessive mutations.

Results: Several new mutations were either true-breeding or segregating in the F_{11} (Table 1). Each of these was recovered in a different F_{11} family. All were derived from germinal revertant F_{10} plants and probably descend from mutational events in germline sectors of mutable F_{9} plants. These mutations are described in more detail in the accompanying research notes.

In addition, possible new mutations were recovered as single variant plants in several other ${\rm F}_{11}$ families (Table 2). If any of these variant plants is the result of a genetic mutation, the mutational event probably occurred in a germline sector of the ${\rm F}_{10}$ parent. Inheritance of these possible new mutations is the subject of current research.

<u>Discussion</u>: We have identified an array of new mutations in the Asgrow Mutable line. Molecular genetic analysis of these materials is underway. If an inserted element is identified at the mutable allele of the w4 locus, it should be possible to clone a molecular probe from the element and analyze the new mutations described in the present study. Cosegregation of DNA polymorphisms homologous to the probe with any of these new mutations would provide proof of transposition of the element in the genome.

Acknowledgment: We thank José Bravo for his assistance at the Asgrow Seed Co. winter soybean nursery at Isabela, Puerto Rico.

Table 1. Summary of new mutations recovered as true-breeding or segregating in $F_{1\,1}$ families of the Asgrow Mutable line

Mutation	Description
Chlorophyll deficient #1	Segregating for normal and variegated chlorophyll pigmentation. Variegated plants have normal and delayed-green sectors.
Chlorophyll deficient #2	Segregating (approximately 3 wildtype:1 mutant) for normal and delayed-green chlorophyll pigmentation.
Chlorophyll deficient #3	Same as Chlorophyll deficient #1.
Partial sterile #1	True-breeding for reduced number of seed per pod.
Partial sterile #2	Segregating for plants producing normal and reduced numbers of seed per pod.
Partial sterile #3	Same as Partial sterile #2.
Partial sterile #4	Same as Partial sterile #2.
Necrotic root #1	Segregating (approximately 3 wildtype:1 mutant) for plants with normal and necrotic root systems.
Necrotic root #2	Same as Necrotic root #1.
Necrotic root #3	Same as Necrotic root #1.

Table 2. Summary of possible new mutations recovered as single plants in $\rm F_{11}$ families of the Asgrow Mutable line

Mutation	Description
Chlorophyll deficient #4	Variegated with normal green and yellow sectors.
Chlorophyll deficient #5	Yellow.
Chlorophyll deficient #6	Green with one yellow unifoliolate.
Chlorophyll deficient #7	Variegated with normal green and delayed-green sectors.
Chlorophyll deficient #8	Variegated with normal green and yellow sectors in one unifoliolate.
Nearly sterile	Almost completely sterile except for several normal pods.

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2) Chlorophyll-deficient mutants in a genetically unstable line of soybean.

Mutations for chlorophyll deficiency were identified in an experiment designed to recover new mutations in the Asgrow Mutable line as evidence for transposition of a mobile genetic element. A detailed protocol for the experiment is presented in the preceding research note (Groose and Palmer, 1987). Each of three independent mutations that are described below was discovered in a sandbench test of a different F_{11} family, each of which had descended from a different F_0 plant of the Asgrow Mutable line.

<u>Chlorophyll deficient #1</u>: In the sandbench test of this F_{11} family, 30 seedlings were normal green (wildtype) and four seedlings were variegated for normal and delayed-green sectors. In newly-opened leaves, chlorophyll-deficient sectors were yellow. As leaves fully expanded, however, deficient sectors became nearly normal green.

In the field nursery, chlorophyll-deficient sectors were not detected on any of 24 plants of this ${\rm F}_{11}$ family. These plants were threshed separately to produce 24 ${\rm F}_{12}$ families and approximately 30 seed of each ${\rm F}_{12}$ family were planted in the greenhouse sandbench. Seedlings were examined frequently for chlorophyll-deficient sectors until the second trifoliolate leaf stage. Overall, 497 (84.1%) seedlings were normal, 73 (12.4%) seedlings were variegated for normal and delayed-green sectors, and 21 (3.6%) seedlings were entirely delayed-green. Although chlorophyll deficiency had not been detected

in any of the 24 field-grown F_{11} parents, chlorophyll-deficient seedlings (either variegated or entirely delayed-green) were found in 19 of the 24 F_{12} progenies. Five F_{12} families produced only normal green seedlings. A chisquaretest for homogeneity of the distribution of normal and chlorophyll-deficient individuals among F_{12} families was performed. Variegated and entirely delayed-green seedlings were considered together as a single class because entirely delayed-green seedlings were relatively rare. The test for homogeneity indicated differential production of normal and chlorophyll-deficient progeny by the F_{11} parents ($\chi^2=123.665$, 23 df, P < 0.005). The mode of inheritance of this unstable trait remains to be determined.

<u>Chlorophyll deficient #2</u>: In the sandbench test of this F_{11} family, 23 seedlings were normal green (wildtype) and nine seedlings were delayed-green. Newly-opened leaves of delayed-green seedlings were yellow but became nearly normal green as leaves fully expanded. No variegated seedlings were observed and it appeared that this family was segregating for a stable recessive mutation. This mutation appears to be deleterious. Although mutant seedlings eventually attained nearly normal chlorophyll pigmentation, they were stunted relative to normal segregates.

In the field nursery, chlorophyll-deficient plants had not been noticed among 31 plants of this F_{11} fmaily. Subsequent to the discovery of the mutation in the sandbench test, a close examination of the field-grown plants indicated that 13 of the 31 plants were slightly paler green and were stunted relative to normal plants of the Asgrow Mutable line. These were classified as "possible mutants" (recessive homozygotes). One of these died before maturity. The thirty plants that survived to maturity were threshed separately to produce 30 F_{12} families.

The F $_{12}$ families were used to test the hypothesis that the F $_{11}$ family was descended from an F $_{10}$ parent that was heterozygous for a new single gene recessive mutation. Approximately 30 seed of most F $_{12}$ families were planted in the greenhouse sandbench. Field-grown plants that had been classified as possible mutants produced less than 30 seed per plant. The sandbench test revealed that five, twenty, and five F $_{12}$ families, respectively, were truebreeding wildtype, segregating, and true-breeding delayed-green. This segregation fit the 1:2:1 ratio expected for F $_{11}$ progeny of an F $_{10}$ parent heterozygous for a single gene mutation (χ^2 = 3.333, 2 df, 0.100 < P < 0.250).

Analysis of segregation within the 20 segregating F_{12} families provided additional evidence that the "chlorophyll deficient #2" is conditioned by a recessive mutation at a single locus. Seedlings in these families segregated at the 3:1 ratio expected for F_{12} progeny of heterozygous F_{11} parents (Table 1).

Table 1. Segregation of seedlings (normal:chlorophyll deficient) within F_{11} -derived F_{12} families segregating for "Chlorophyll deficient #2"

	Normal	Chlorophyll deficient	χ ² (3:1)	P
Totals	357	105	16.882	
Pooled chi-square (ldf)			1.273	.2550
Homogeneity chi-square (19 df)			15.549	.5075

All five F_{12} families that were true breeding for delayed-green were descended from F_{11} plants that had been classified as "possible mutants." The other surviving F_{11} "possible mutants" proved not to be recessive homozygotes. Thus, field evaluation of this trait proved to be somewhat unreliable. Recessive homozygotes were always pale green and stunted but the converse was not always true.

<u>Chlorophyll deficient #3</u>: In the sandbench test of this F $_{11}$ family, 16 seedlings were normal green (wildtype) and eight seedlings were variegated for normal and delayed-green sectors. Variegated seedlings were identical to variegated seedlings of "Chlorophyll deficient #1" except that delayed-green sectors tended, on average, to be larger than delayed-green sectors produced on variegated plants of "Chlorophyll deficient #1." For example, entirely delayed-green unifoliolate leaves were produced on several variegated plants.

In the field nursery, chlorophyll-deficient sectors were not detected on any of 16 plants of this F_{11} family. These plants were threshed separately to produce $16\ F_{12}$ families which were studied in the same way as the F_{12} families of "Chlorophyll deficient $\sharp 1.$ " Overall, 359 (90.9%) seedlings were normal, 26 (6.6%) seedlings were variegated for normal and delayed-green sectors, and 10 (2.5%) seedlings were entirely delayed-green. Although chlorophyll deficiency had not been detected in any of the 16 field-grown F_{11} parents, chlorophyll-deficient seedlings were found in 9 of the 16 F_{12} progenies. Seven F_{12} families produced only normal green seedlings. The chi-square test for homogeneity of the distribution of normal and deficient individuals among F_{12} families indicated differential production of normal and deficient progeny by the F_{11} parents ($\chi^2=72.300$, 15 df, P < 0.005). Like "Chlorophyll deficient $\sharp 1$," the mode of inheritance of this unstable trait remains to be determined. It is interesting that these three mutations for chlorophyll deficiency are all characterized by a delayed-green phenotype. We are currently entertaining the hypothesis that these are three independent mutations (one stable and two unstable) of the same nuclear gene.

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3) Partially sterile mutants in a genetically unstable line of soybean.

Mutations for partial sterility were identified in an experiment designed to recover new mutations in the Asgrow Mutable line as evidence for transposition of a mobile genetic element (Groose and Palmer, 1987). The mutations were discovered in a field nursery that included 1697 F_{11} progeny rows of the Asgrow Mutable line. Mutant plants with reduced numbers of seeds per pod were first detected when the nursery was examined at maturity. Each of the four independent mutations for partial sterility was discovered in a different F_{11} family, each of which had descended from a different F_{9} plant of the Asgrow Mutable line.

Normal plants of the Asgrow Mutable line produce mostly three— and two-seeded pods. A random sample of plants taken from throughout the nursery produced one—, two—, three—, and four—seeded pods as indicated in Table 1. Plants of a distinctly different type were found in four ${\rm F}_{11}$ families. These plants were characterized by a reduced number of seeds per pod and were designated "partial steriles." Partial sterile plants produce a preponderance of one— and two—seeded pods (Table 1) and are easily recognized at maturity by the large number of one—seeded pods. Partial sterile plants were otherwise identical to normal plants. The reduction in number of seeds per pod appeared to be the result of ovule or very early embryo abortion.

Table 1. Number and percentage of four different types of pods produced by normal and partial sterile mutant ${\rm F}_{11}$ plants of the Asgrow Mutable line

		Type of pod							
	No.		e- ded		o- ded		ee-	Fou	ır- ded
Entry	plants	No.	%	No.	%	No.	%	No.	%
Asgrow Mutable line ^a	16	118	10.6	466	41.8	521	46.7	10	0.9
Partial sterile #1 ^b	12	514	44.1	511	43.9	139	11.9	1	0.1
Partial sterile #2 ^c Normal plants Partial steriles	9 11	84 423	12.6 47.1	276 388	41.5 43.2	296 87	44.5 9.7	9	1.4
Partial sterile #3 ^C Normal plants Partial steriles	3 12	35 507	14.5 50.3	99 418	41.1 41.5	105 82	43.6 8.1	2	0.8 0.1
Partial sterile #4 ^C Normal plants Partial steriles	8 12	50 335	10.4 48.6	176 293	36.5 42.5	244 60	50.6 8.7	12	2.5 0.1

^aRandom sample of F₁₁ plants from throughout the nursery.

All plants in the F_{11} family in which "Partial sterile #1" was discovered were partial steriles. The families in which "Partial sterile #2," "Partial sterile #3," and "Partial sterile #4" were discovered were segregating for normal and partial sterile plants. Normal plants in these families produced one-, two-, three-, and four-seeded pods in essentially the same proportions as the random sample of plants taken from throughout the nursery. Partial sterile plants in these families produced one-, two-, three-, and four-seeded pods in essentially the same proportions as plants of "Partial sterile #1." The mechanism and inheritance of partial sterility in these materials is the subject of current research.

All plants in this F11 family were partial sterile.

 $^{^{\}mathrm{C}}$ This F_{11} family was segregating for normal and partial sterile plants.

Reference

Groose, R. W. and R. G. Palmer. 1987. New mutations in a genetically unstable line of soybean. Soybean Genet. Newsl. 14: 164-167.

R. W. Groose - USDA R. G. Palmer - USDA

4) Necrotic root mutants in a genetically unstable line of soybean.

Mutations for necrotic roots were identified in an experiment designed to recover new mutations in the Asgrow Mutable line as evidence for transposition of a mobile genetic element (Groose and Palmer, 1987). Each of three independent mutations for necrotic roots was discovered in a different ${\tt F}_{11}$ family, each of which had descended from a different Fg plant of the Asgrow Mutable line.

Seedlings of 1936 F_{11} families were pulled from the sandbench at the second trifoliolate leaf stage and roots were examined for nonfluorescent root mutations. Seedlings of most soybean lines, including the Asgrow Mutable line, fluoresce under UV light. Root fluorescence is conditioned by several loci (Sawada and Palmer, 1987). Putative nonfluorescent mutants were identified in three F_{11} families. However, close examination of the mutants revealed that the failure of the roots to fluoresce was not the result of mutation at any of the loci for root fluorescence. The apparent lack of fluorescence in these mutants was due to an accumulation of a reddish-brown slime that obscured an underlying fluorescent root surface. The root systems of these mutants were weak and necrotic. Several mutants were transplanted to pots in the greenhouse but eventually most died. Survivors produced very few progeny. It is unlikely that plants with this trait would survive to maturity in the field.

In the sandbench test of the three F_{11} families, seedlings segregated approximately 3 wildtype:l necrotic (Table 1). We hypothesized that each of the F_{11} families was segregating for a new recessive mutation for necrotic roots and tested the hypothesis with F_{11} -derived F_{12} families. For each of the three F_{11} families, plants that had been grown in a field nursery were threshed separately and their F_{12} progenies were tested in the sandbench. In each case, approximately one-third of F_{12} families were true-breeding for normal roots and approximately two-thirds of F_{12} families were segregating for normal and necrotic root (Table 2). In each case, the observed segregation fit the 1:2 ratio expected for F_{11} progeny of an F_{10} parent heterozygous for a lethal recessive mutation.

Analysis of segregation ratios within F_{12} families provided additional insight into the behavior of the necrotic root trait. F_{12} families derived from heterozygous F_{11} parents are expected to segregate 3 normal:1 necrotic in the sandbench test. For all three mutations, a small excess of seedlings was observed in the necrotic root class (Table 3). In the case of "Necrotic root #3", the deviation from the expected 3:1 was significant at the P=0.01 level. At present, we believe that the excess in the recessive class is easily explained as the result of misclassification. In the sandbench, a proportion of seedlings of any soybean line will die from a variety of causes.

Roots of such seedlings may mimic the necrotic root trait. In this experiment it is likely that some seedlings that were not homozygous recessive for the necrotic root trait were misclassified as "necrotic root," In other words, classification for this trait is not expected to be perfectly reliable and a slight deviation from the 3:1 might be expected. On the whole, we believe that the results of this experiment support the conclusion that "Necrotic root #1," "Necrotic root #2," and "Necrotic root #3" each resulted from a recessive mutation at a single locus. Genetic analysis is in progress to determine if these three independent mutations are allelic to the same locus.

Table 1. Segregation of seedlings (normal root:necrotic root) in three F $_{\hbox{\scriptsize 11}}$ families of the Asgrow Mutable line

	Necrotic root #1		Necroti	c root #2	Necrotic root #3		
	Normal	Necrotic	Normal	Necrotic	Normal	Necrotic	
F ₁₁ seedlings	37	12	21	7	33 ·	12	
Chi-square (3:1, 1df)	0.007		0.000		0.067		
Probability	0.90-0.95		0.995-1.000		0.75-0.90		

Table 2. Ratio of F_{11} -derived F_{12} families (true-breeding normal:segregating) for three necrotic root mutations recovered in the Asgrow Mutable line)

	Necrotic root #1		Necrotic	root #2	Necrotic root #3		
	True breeding normal	breeding Segre-		True breeding Segre- normal gating		True breeding Segre- normal gating	
F ₁₂ families	4	15	8	10	6	11	
Chi-square (1:2, ldf)	1.28	1.286		1.000		0.029	
Probability	0.25-0	0.25-0.50		0.25-0.50		0.75-0.90	

Table 3. Segregation of seedlings (normal root:necrotic root) within segregating F_{11} -derived F_{12} families for three necrotic root mutations recovered in the Asgrow Mutable line

	Necrotic	root #1	Necrotio	root #2	Necrotio	root #3
	Normal	Necrotic	Normal	Necrotic	Normal	Necrotic
Total F ₁₂ seedlings	260	103	197	78	214	98
% F ₁₂ seedlings	71.6	28.4	71.6	28.4	68.8	31.4
Pooled chi-square	2.2	27	1.6	560	6.8	337
(3:1, ldf) Probability	0.10-	0.25	0.10-	-0.25	0.005	5-0.010
Homogeneity chi-square	13.356	(14df)	17.871	(9df)	11.183	(10df)
Probability	0.25-	0.50	0.025	5-0.050	0.25-	-0.50

Groose, R. W. and R. G. Palmer. 1987. New mutations in a genetically unstable line of soybean. Soybean Genet. Newsl. 14:164-167.

Sawada, S. and R. G. Palmer. 1987. Genetic analysis of nonfluorescent root mutants induced by mutagenesis in soybean. Crop Sci. 27:62-65.

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R. G. Palmer - USDA

5) Monosomics from synaptic KS mutant.

Our study of unknown synaptic mutants attempts to provide more information about genetics of megasporogenesis and microsporogenesis in soybean. This study attempts also to develop aneuploid stocks to be used to establish a genetic map in soybeans. Synaptic mutants are known to produce aneuploid and polyploid plants. We believe that variability in genetic background of new synaptic mutants can be helpful in obtaining all 20 possible primary trisomics and other aneuploids in soybeans.

<u>Materials and methods</u>: Sterile plants were found in the F3 progeny from a cross between two dwarf plants (T210 df2 x dwarf mutant 35-1-2) from material obtained from K. Sadanaga. F3 progeny segregated 41 fertile to 15 sterile plants. Forty F_4 KS progenies were observed for sterility vs. fertility segregation in 1985. For aneuploid identification, the method described by Palmer and Heer (1973) was used for chromosome number determination. I_2 KI stain was used for estimation of pollen fertility/sterility.

Results and discussion: Among 40 F_4 progenies, 13 did not segregate; 27 progenies segregated for sterility. Among segregated progenies, 915 plants were fertile and 330 plants were sterile (Table 1). These results indicated that a single locus was conditioning female and male sterility.

Table 1. Segregation for fertility vs. sterility in ${\rm F}_4$ population of KS synaptic mutants

ed number of p	lants	Expected numb	er of plants
Sterile	Total	Fertile	Sterile
330	1245	933.75	311.25
53			
25 - 0.10			
	Sterile 330 53	330 1245	Sterile Total Fertile 330 1245 933.75 53

KS mutation was highly female and male sterile. Only ten seeds were harvested from all sterile plants (0.03 seed per plant). Nine seeds germinated and seedlings were checked for chromosome number. In the progeny of sterile plants, diploids, addition aneuploids, deficiency polyploids and, what was the most valuable, a deficiency aneuploid 39-chromosome plant, No. KS-6, were identified (Table 2).

Original monosomic - KS-6: The monosomic was grown during winter/spring seasons in the USDA greenhouse. We noticed partial sterility of this plant, 24.7% nonviable pollen grains (Table 3). As far as the female reproduction is concerned, the number of abortions was higher than number of harvested seeds. Especially high frequency of ovule abortion was noted (Table 3). This original monosomic produced 130 seeds.

Table 2. Chromosome number of progeny of the KS sterile mutant

Entry No.	No. of seeds	No. of chromosomes	Fertility
S85-22	1	44	Fertile
S85-26	1	42	Fertile
S85-28	1	44	Died
S85-29	1	40	Fertile
S85-32	1	39	Partially sterile
S85-33	2	45	Fertile
		72	Sterile
S85-34	1	41	Sterile
S85-37	1	43	Sterile

Table 3. Fertility of the original 39-chromosome plant KS-6

Traits	Number	Percentage
Stained pollen grains	870	75.3
Nonstained pollen grains	285	24.7
Number of seeds	130	41.1
Number of abortions	186	58.9
Aborted embryos	6	
Aborted ovules	180	
Ratio seeds:abortions	0.7	
Ratio of aborted embryos:ovules	0.03	

Progeny of original monosomic KS-6: Of the 1380 seeds, 94 germinated; their chromosome numbers were determined. Ninety-two seedlings were diploids, two seedlings, KS-6-21 and KS-6-26 (Fig. 1), had 39 chromosomes. Transmissions of n-1 gametes was very low; frequency of monosomics was 2.1%. We did not find any 2n + 1 genotypes. KS-6-21 and KS-6-26 plants were grown during summer 1986 in the greenhouse. Plant KS-6-21 had only 3% sterile pollen grains; plant KS-6-26 had 17.1% sterile pollen grains (Table 4). From a comparison of pollen fertility and seed set in several species, abnormalities of female reproduction were considered to be less than on the PMC side. However, in some species (Oriza sativa, Brassica oleracea var. capitata), a stronger effect of factors causing disturbances in cell division was observed on megasporogenesis than on microsporogenesis (Katayana, 1964; Gottschalk and Konvicka, 1971). In observed monosomics, the percentage of abnormalities on the female side was higher than on the male side. Low transmission of n-1gametes from the original monosomic, high rate of abortions of KS-6-21 and of KS-6-26 make these plants unreliable as sources of monosomics by sexual reproduction.

Soybeans can tolerate addition aneuploids and set seeds. Beversdorf and Bingham (1975) found addition aneuploids among the normally-shaped seeds of 40-chromosome plants derived from 43-44-chromosome plants. A few trisomics



Figure 1. Metaphase of 39-chromosome soybean, KS 6-26.

Table 4. Fertility of 39-chromosome plants derived from KS-6

Traits	KS-6-21	KS-6-26
Stained pollen grains	490	1062
Nonstained pollen grains	15	219
Percentage of sterile pollen grains	3.0	17.1
Number of seeds	108	121
Number of abortions	91	105
Percentage of abortions	45.7	46.5
Number of aborted embryos	35	42
Number of aborted ovules	56	63
Ratio seeds:abortions	1.2	1.1
Ratio seeds:ovules	0.6	0.7

were obtained by using haploids, but no monosomics or other deficiency aneuploids have been confirmed (Sorrels and Bingham, 1978). In chromosome transmission of n + 1 gametes, soybeans behave similarly to known polyploids. Ovule transmission averages 34% for Tri A, 45% for Tri B, 39% for Tri C, and pollen transmission rates were respectively 27%, 22%, and 43% (Palmer, 1972). It is difficult to find an explanation why viable n-1 gametes are so rarely produced.

A system found in corn produces a high frequency of monosomics. They are generated by r-Xl deficiency, a submicroscopic deficiency including the R locus on chromosome 10 (Weber 1970, 1973, 1982). To establish a genetic map of soybeans, we have to search for an efficient system of obtaining monosomics, as well as to search for new translocations.

In our study, monosomics were fertile or partially sterile. Progeny of the original monosomic segregated for fertility vs. sterility and gave a good fit to 3:1 ratio (Table 5). These observations showed that the KS sterile mutation is not located on the missing chromosomes.

Table 5. Segregation for fertility vs. sterility of the progeny of original monosomic KS-6 $\,$

0bs	erved number of pl	ants	Expected number	r of plants
Fertile	Sterile	Total	Fertile	Sterile
68	24	92	69	23
χ ² (3:1)	0.057			
P	0.90 - 0.75			

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6) New male-sterile, female-fertile mutations in soybeans.

Three new spontaneous independent male-sterile, female-fertile (MS-FF) mutations were used for cytological observations and for allelism tests. One of them was found in the cultivar 'Beeson'. The JB and BDI mutations were found in advanced breeding populations. The Beeson, JB, and BDI mutants produced sterile plants, which at the time of anthesis have shrunken, distorted anthers. These sterile plants can be differentiated easily from fertile plants by examining the flowers with magnifying glasses. Degenerated microspores were not released from anthers. Male sterile-female fertile mutant ms2 ms2 has similar anther features.

 $\underline{\text{Methods:}}$ Beeson, JB, and BDI mutants were crossed with ms2 genotypes. We made crosses among the new mutants as well. In most crosses, sterile plants were used as female parents and known heterozygotes were used as male parents. In cross number 1 (Table 1) and number 5 (Table 2), genotypes of male parent were unknown. They could be dominant homozygotes or heterozygotes of sterility genes.

Table 1. Fertility of F1 plants from crosses of unknown male-sterile, femalefertile mutants with the ms2 genotype

Cross		Number of	plants
		Fertile	Sterile
1.	ms ms BDI x Ms2 Ms2, Ms2 ms2	46	0
2.	ms2 ms2 x Ms ms BDI	20	0
3.	ms ms Beeson x Ms2 ms2	46	0
4.	ms ms JB x Ms2 ms2	28	0
5.	ms2 ms2 x Ms ms JB	17	0

Table 2. Fertility of F_1 plants among unknown male-sterile, female-fertile mutants

		Number of plants		
	Cross	Fertile	Sterile	
1.	ms ms Beeson x Ms ms BDI	49	0	
2.	ms ms JB x Ms ms BDI	45	. 0	
3.	ms ms BDI x Ms ms JB	10	0	
4.	ms ms Beeson x Ms ms JB	13	0	
5.	ms ms Beeson x Ms Ms JB, Ms ms JB	16	0	
6.	ms ms JB x Ms ms Beeson	37	0	

 $\rm F_{1}$ seeds were planted in the Iowa State University-University of Puerto Rico Soybean Breeding Nursery, Isabela, Puerto Rico. During flowering, buds were collected for pollen viability evaluation.

Results and discussion: We obtained sufficient number of seeds from test crosses to get information about allelism of the new mutations.

 F_1 plants from crosses of BDI, Beeson, and JB mutations with $_{\it ms2}$ genotype were fertile, indicating that none of these new mutations are located at the $_{\it ms2}$ locus.

 $F_{\rm I}$ plants from crosses made among new mutations also were fertile, indicating that they are non-allelic to each other.

It is interesting that in soybeans one locus (ms1 locus) has six independent mutations (Skorupska and Palmer, 1986), the ms3 was found to have two mutations (Graybosch and Palmer, 1987), and, conversely, different alleles can produce the same phenotypic effect but are located at different loci.

Our observations have shown that the JB mutation is closely linked (2.48-3.25% of recombination) to the wI locus (Skorupska and Palmer, 1986). We crossed the BDI, Beeson and JB mutants with the satellite trisomic. The purpose of this aspect of our study was to find if new MS-FF mutations are located in linkage group 8. This material is now under investigation. We can expect positive results as far as the JB mutant is concerned, because the wI gene is on the satellite chromosome (Sadanaga and Grindeland, 1984). If data show that BDI and Beeson mutations are not in LG8, we will be able to avoid testing of locus-to-locus linkage with genes located in LG8.

References

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7) New independent mutation: ms1 (Ames 2).

Five different populations have been recognized as a source of ms1 alleles. Genetics studies of male-sterile, female-fertile mutations conducted by Palmer et al. (1978) showed that ms1 (North Carolina) (T260), ms1 (Urbana) (T266), ms1 (Tonica) (T267), and ms1 (Ames) (T268) are independent mutations at the ms1 locus. Yee and Jian (1983) reported another mutation at the ms1 locus, designated Shennong Male-Sterile Soybean L-78-387.

Sterile plants setting very few pods were found by Ron Secrist in his plant breeding nursery in 1984. Our previous observations based upon ${\rm F_1}$ allelism tests indicated that this unknown mutant was allelic to ms1 (T266) (Skorupska and Palmer, 1986). This report reviews the origin of the mutant and gives ${\rm F_1}$ and ${\rm F_2}$ results.

Origin of unknown mutant. Plant breeding material was derived from 1979 to 1984 from 100 seeds of the Soybean Germplasm Population, AP6 (S1) C1 (see Figure 1, Skorupska and Palmer, 1986). According to Ron Secrist, $msl\ msl$ plants have never been grown in his nursery and the populations had no chance for contamination with the msl allele.

Methods. Crosses were made in 1985 using homozygous recessive ms1 ms1 (T266) plants as female parents and heterozygotes of the new mutant as male parents. F_1 seeds were planted in the ISU Soybean Breeding Nursery in Isabela, Puerto Rico, and in the USDA greenhouse in Ames. Thirty-eight F_1 plants were obtained for allelism tests. They were classified for male sterility/fertility on the basis of pollen staining in I₂KI (Table 1). F_1 progenies were grown in Ames in 1986 and plants were classified at maturity for sterility/fertility segregation. Sterile plants of ms1 ms1 (T266) and the unknown mutant were observed for their pod-set under field conditions in 1985 and 1986.

Results. In the F_1 generation, 20 plants had normal pollen, 18 plants were characterized by large coenocytic pollen grains. This population gave a good fit to the expected 1:1 ratio, χ^2 = 0.106, P = 0.75-0.50 (Table 1.) Twenty F2 progenies segregated for sterility at maturity. In the F_2 , 1,400 plants were fertile and 455 were sterile. The data gave a good fit to the expected 3:1 ratio and confirmed results of testcrosses in the F_1 generation (Table 1). Results indicated that independent mutation has occurred at the ms1 locus.

Pod set observed on sterile plants of $ms1\ ms1\ (T266)$ and plants of the new mutation suggested that the new mutation was not as female fertile as $ms1\ (T266)$. During two years of observations, plants of $ms1\ ms1\ (T266)$ averaged 2.0 seed per pod and give 13.2 seeds per plant. The mutant averaged 1.1 seed per pod and 4.4 seeds per plant.

We propose to name strain T268 as ms1 (Ames 1) and strain carrying new mutation as ms1 (Ames 2) (T287).

Table 1. Segregation for fertility/sterility in F $_1$ and F $_2$ population from cross $ms1\ ms1\ (T266)$ x $Ms1\ ms1$ unknown mutant

Number of plants				Expected		
Generation	Fertile	Sterile	Total	ratio	χ ²	P
F ₁	20	18	38	1:1	0.106	0.70-0.50
F ₂	1400	455	1855	3:1	0.220	0.70-0.50
Total (20 df))				5.075	>0.995
Homogeneity)	χ ² (19 df)				4.855	>0.995

Table 2. Seed production on sterile plants of new mutant and ms1 ms1 (T266)

		Number of sterile	f sterile — Average number		
	Year	plants	Seeds/plant	Seeds/pod	
New mutant	1985 1986	174 100	5.9 3.0	1.1 1.1	
	1900	100	4.84	1.1	
ms1 ms1	1985	32	14.9	2.1	
(T266)	1986	50	11.6 12.89	$\frac{2.0}{2.0}$	

Palmer, R. G., C. L. Winger and M. C. Albertsen. 1978. Four independent mutations at the *ms1* locus in soybeans. Crop Sci. 18:727-729.

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8) Inheritance and derivation of T218H.

Genetic type T218M was found in the cultivar 'Illini' in 1952 at Urbana, Illinois. Genetic type T225M was found in the cultivar 'Lincoln' in Iowa before 1955. T218M phenotypically is similar to T225M in that an unstable allele results in a chlorophyll chimera. We derived T225H (Y18 y18) by crossing Lincoln as female parent by T225 a yellow plant (y18 y18) grown in the growth chamber, as male parent (Sheridan and Palmer, 1975). We also have transmitted the y18 gamete in crosses by using flowers from a yellow branch on a variegated plant (Sheridan and Palmer, 1975). Our objective was to develop T218H.

Both T225H and T218H give predictable 3:1 phenotypic ratios and 1:2:1 genotypic ratios, upon self-pollination of heterozygotes. The recessive genotype is lethal under field conditions and is a conditional lethal in the greenhouse. Heterozygous plants are used in cross-pollinations in genetic studies.

We used Illini as the female parent and flowers from a mostly yellow branch on a variegated T218M plant as male parent. All nine $\rm F_1$ plants were green as expected. In the $\rm F_2$, two progenies were all green. Seven progenies segregated about 3 green:1 yellow plants (Table 1). Twenty $\rm F_2$ plants within each of these seven families were threshed individually and were evaluated as $\rm F_2$ -plant-progeny rows. About two-thirds of the 140 entries segregated, as expected, for green and yellow plants (Table 1). The ratio of green:yellow plants in these segregating families was about 3:1 (Table 1).

The failure of all nine F_1 plants to segregate in the F_2 for green and yellow plants was not unexpected. In plants, in general, segregation patterns of chimeras manifest a relationship between sector phenotype and gamete genotype. When chimeric plants are used in crosses and the yellow trait is a single-gene recessive 1) flowers borne on nonchimeric green regions produce all green F_2 progeny, 2) flowers borne on sectored regions may produce all green progeny, green and variegated progeny, or about 3 green:1 yellow progeny, and 3) flowers borne on yellow regions produce all segregating progeny of about 3 green:1 yellow.

Hatfield and Palmer (1986) tested for allelism between T218H and T225H. F_1 , F_2 , and F_3 data reported by them confirmed the allelism of these two mutants. On the basis of these results, the heterozygote should be added to the Genetic Type Collection as T218H and the appropriate gene symbol should be Y18 y18.

Table 1. Inheritance and derivation of T218H. Parents were Illini x T218M

H .	No. F2	No. F ₂ plants	× ×	THE STATE OF THE S	F ₂ families	lies	× ×		No. F3 plants seg. families	o. F3 plants in seg. families	× ×	
plant no.	Green	Yellow		A A	Nonseg./Seg	/Seg.	(1:2)	Д	Green	Yellow	(3:1)	Д
-	319	101	0.20	0.50-0.75	10	10	2.50	0.10-0.25	3201	1097	0.64	0.25-0.50
2	297	87	1.12	0.25-0.50	2	15	0.62	0.25-0.50	4455	1374	6.34	0.01-0.025
3	310*	0	0	0	20*	0	0	0	0	0	0	0
4	197*	0	0	0	20*	0	0	0	0	0	0	0
5	185	99	0.40	0.50-0.75	4	16	1.59	0.10-0.25	4931	1603	0.76	0.25-0.50
9	483	152	0.38	0.50-0.75	9	14	0.10	0.75	4713	1529	0.85	0.25-0.50
7	512	165	0.14	0.50-0.75	7	13	0.03	> 0.90	4127	1331	1.09	0.25-0.50
∞	403	133	0.01	06.0<	9	14	0.10	0.75	4544	1503	0.07	0.75-0.90
6	369	119	0.10	0.75	7	13	0.03	>0.90	4380	1417	1.10	0.25-0.50
Total	2568	813	2.35	>0.90	45	95	4.97	0.75-0.50	30,351	9854	10.85	0.10-0.25
Pooled (1 df)			1.64	0.10-0.25			0.10	0.75			5.16	0.01-0.025
Homogeneity (6 df)	neity		0.71	>0.90			4.87	0.50-0.75			5.69	0.25-0.50

*Not included in total.

Table 2. Description of T218M, T218H, T225M, and T225H

Strain	Gene	Phenotype	Source
T218M	Y18-m	Chlorophyll chimera (resembles T225M)	Found in Illini in 1952
Т218Н	y18	Near-lethal, yellow leaves	From T218M
T225M	Y18-m	Unstable allele resulting in chlorophyll chimera	Found in Lincoln in Iowa before 1955
Т225Н	y18	Near-lethal, yellow leaves	From T225M

Sheridan, M. A. and R. G. Palmer. 1975. Inheritance and derivation of T225, Y18 y18. Soybean Genet. Newsl. 2:18-19.

Hatfield, P. M. and R. G. Palmer. 1986. Allelism tests of T218H and T225H. Soybean Genet. Newsl. 13:147-149.

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9) PI 153252 is a true-breeding yellow mutant.

We have searched for chromosome interchanges and inversions among progeny of accessions crossed with cultivars of standard chromosome structure. We make the cross-pollinations and examine the F_1 plants for fertility/sterility (Delannay et al., 1982; Palmer et al., 1987). If sterility is present, meiocytes are examined to confirm the presence of a chromosome aberration. We routinely plant about 50 F_2 seed from each F_1 plant to check segregation of marker genes to confirm hybridity.

In the cross of 'Swift' x PI 153252, the two F_1 plants were fertile. In the F_2 , green plants and yellow plants were observed among progeny from both F_1 plants (Table 1). The yellow plant color became more evident in the new leaves when the plants were growing vigorously and the ambient temperature was increasing. We are not certain whether the yellow color of the emerging leaves was the result of plant age (developmental stage), ambient temperature, or their interaction. The yellow plants were quite vigorous.

Sixty-one F_2 plants from one F_1 family (A83-83-2) were chosen at random to advance to the F_3 . Segregation for plant color in the F_3 fit a 1:2:1 ratio for all green:segregating : all yellow (Table 2). Allelism tests with PI 153252 yellow plants with known yellow mutants were not done.

Table 1. F₂ segregation for plant color of Swift x PI 153252

	No. F ₂	plants	2	
F_1 plant no.	Green	Yellow	X 3:1	Р
A83-83-1	267	83	0.31	0.75-0.50
A83-83-2	325	114	0.22	0.75-0.50

Table 2. F_2 genotypes determined from F_3 plants of Swift x PI 153252

	No. F ₂ families		2	
All green	Segregating	All yellow	X 3:1	P
15	31	15	0.02	0.99

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10) Chloroplast DNA analysis of cyt-Y3.

Introduction: The cytoplasmically inherited yellow foliar mutant, cyt-Y3, is the third uniparentally inherited mutant reported for soybeans (Shoemaker et al., 1985). The remaining two maternally inherited mutants are cyt-Y2, also a yellow foliar mutant (Palmer and Mascia, 1980), and cyt-G1, a green cotyledon mutant (Terao, 1918). cyt-Y3 is unique in the respect that it is near-lethal under normal light conditions and lethal under elevated photosynthetic photon flux densities, i.e., 600 to 2,100 microeinsteins m $^{-2}$, respectively.

Uniparentally inherited mutations are difficult to evaluate by classical methods. Because soybean organelles are inherited predominantly, or solely, through the maternal parent (Sisson et al., 1978; Hatfield et al., 1985), the associated genes are inherited as a unit and no recombinational studies are possible. In fact, it is not yet possible to determine unambiguously whether the uniparentally inherited mutation (cyt-Y3) occurs within the chloroplast genome or the mitochondrial genome. Because the cyt-Y3 mutation affects the chloroplast chlorophyll content and chloroplast ultrastructure, we postulate that the mutation lies within the ctDNA molecule.

The mutant, cyt-Y3, is assigned the Laboratory Genetic Type Collection Number T278. The mutant arose as a chimera. Subsequent selfings produced progeny that were yellow (cyt-Y3), green (cyt-G3), and chimera. Consequently, cyt-G3 and cyt-Y3 can be considered genetically identical with the exception of the mutation giving rise to the cyt-Y3 phenotype. Therefore, any DNA sequence variation detected between plant populations expressing the two different phenotypes would indicate the presence of a molecular lesion responsible for the mutant phenotype and would provide a "tag" by which we might identify the location of the coding sequence of the altered gene.

The objective of the experiments described was to attempt to identify ctDNA sequence variation, using restriction endonucleases, between cyt-G3 and cyt-Y3 sib populations. Restriction endonucleases are enzymes that recognize and cleave DNA at specific sequences of four to seven base pairs in length. DNA can be digested with a particular restriction endonuclease, and the mixture of DNA fragments generated then separated by agarose gel electrophoresis. A characteristic pattern of bands can be visualized on the gel. Each band represents a length of DNA between restriction sites. On the basis of comparative electrophoretic mobilities, the number of base pairs constituting each fragment can be estimated. A restriction enzyme cleavage site can be considered a genetic marker and can be used to locate mutant lesions along a chromosome.

Materials and methods: Seed for the cyt-Y3 and cyt-G3 comparisons was increased in field plots in Ames, Iowa during summer, 1986. Due to the lethality of the cyt-Y3 phenotype under normal environmental conditions, seed was increased only from the chimera plants. The yellow plants died as seedlings and green plants were rogued. Bulked seed from chimera plants was grown in the greenhouse sandbench during winter 1986-87 without supplemental lighting.

Trifoliates were harvested from green plants and from yellow plants. Enrichment of ctDNA was carried out according to published procedures (Shoemaker et al., 1984).

ctDNA was digested with the restriction endonucleases HpaII, ClaI, AvaI, XhoI and PstI. DNA fragments were electrophoresed in 0.8% agarose gels in a buffer of 90 mM Tris, 90 mM boric acid, and 2.5 mM Na $_2$ EDTA. Electrophoresis was conducted at room temperature for 18 hr at 50 v. Gels were stained in ethidium bromide solution (0.5 $\mu\text{g/ml})$ and photographed with a MP-4 camera apparatus over short-wave ultraviolet light using Polaroid Land Pack film Type 665 with Kodak Wratten gelatin filters No. 1A (U.V.) and No. 15 (orange).

Results and discussion: Deletions, insertions and other chromosomal rearrangements can alter the relative position of restriction sites, yielding shorter or longer fragments. A loss of a restriction site can occur due to a point mutation within the restriction site or from any chromosomal rearrangement resulting in an alteration of a recognition sequence. An increase in the number of restriction sites may also be observed as a consequence of insertions or rearrangements or base changes resulting in the creation of additional recognition sequences. These differences are reflected as alterations in the pattern of bands produced by gel electrophoresis, and are termed restriction fragment length polymorphisms, or RFLP's.

No RFLP's were observed between ctDNA prepared from cyt-Y3 plants and cyt-G3 plants using the restriction enzymes listed above. If, however, we make the assumption that the mutation responsible for the cyt-Y3 phenotype does lie within the chloroplast genome, the failure to identify an RFLP still provides us with some valuable information. This suggests that the mutant phenotype arises either from a simple point mutation that is not detected with the restriction endonucleases used in this study, or from a relatively small addition, deletion, translocation or inversion that does not involve a restriction site. Studies of cyt-Y3 are continuing in our laboratory by utilizing additional restriction endonucleases.

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1) Cultivar identification by isozyme analysis.

The purpose of this survey is to build a multiple enzyme system which may provide many biochemical characters to fingerprint cultivars. A total of 751 lines and cultivars of *Glycine max* have been screened for their enzyme zymogram types by using the technique of slab-gel electrophoresis (see Chiang, 1985; Doong, 1986; Gorman, 1983; Gorman and Kiang, 1977, 1978; Kiang and Gorman, 1983, for method). For 367 named cultivars, the zymogram types of eight enzymes and one seed protein (Ti) are listed in Table 1. Except for several cultivars, two of the eight enzymes, ADH and LAP, listed in Table 1 were mainly classified by Gorman (1983). The other six enzyme systems were examined by the authors. For 93 T-strains, 51 FC-strains, 208 plant introductions, 20 Korean cultivars and 11 AV strains from Taiwan, the seeds were examined for 16 enzymes and one seed protein (Ti). The zymogram types for each enzyme of these lines or cultivars are listed in Table 2.

The zymogram types and the genetic model(s) for each isozyme studied in this survey are presented briefly in the following sections.

Aconitase (ACO). Ten different homozygous zymogram types, numbered 1 through 10, were observed in the G. max germplasm (Doong, 1986; Doong and Kiang, 1987). Six bands of aconitase activity, five of which exhibited mobility variants or apparent null alleles, were evident (Fig. 1). Type 3 was the most common, occurring in 456 lines and cultivars. Band 6 at the Rf 0.55 position in type 5 and the Rf 0.49 position in all other types, was vague on the gel and, therefore, was named Band 6.

Based on the fact that F_1 genotypes and F_2 segregation ratios were the same for the reciprocal crosses, Doong (1986) reported that band 2 (Rf's 0.35 or 0.42) and band 4 (Rf's 0.61, 0.63 or 0.69) isozymes were coded by nuclear genes. Genetic study showed that band 2 and band 4 were the products of two unlinked loci, Aco2 and Aco4, with two (Aco2-a and Aco2-b) and three (Aco4-a, Aco4-b and Aco4-c) codominant alleles, respectively (Doong, 1986; Doong and Kiang, 1987). Heterozygotes displayed both parental band sets without any intermediate band, indicating a monomeric structure for aconitase.

Alcohol dehydrogenase and Aldolase (ADH & ALD). Three homozygous ADH zymogram types were reported occurring in the cultivated and wild soybeans (Gorman and Kiang, 1977; Gorman, 1983; Kiang and Gorman, 1983). These three homozygous types are shown in Fig. 2. Both types 1 and 2 had five bands, for type 1, bands 1, 3, 4, 6 and 7; for type 2, bands 2, 3, 5, 6 and 7. Type 3 had four bands, bands 2, 3, 6 and 7. Chiang (1985) reported that the soybean ADH had a dimeric structure. Bands 1 and 7 were controlled by two different loci, and band 4 was their interlocus band (heterodimer). The genetic controls of ADH also have been reported by other investigators (Gorman and Kiang, 1978; Gorman, 1983; Kiang and Gorman, 1983).

The zymogram types of aldolase, following the staining methods of O'Malley et al. (1980), Shaw and Prasad (1969), and Vallejos (1983), were found to be the same as those of alcohol dehydrogenase among 367 named cultivars studied (see Table 1). Doong (1986) suspected that all the "ALD bands" observed are

not ALD bands but ADH bands. The possible explanation is that the substrate in the staining solution, fructose-1,6-diphosphate, was converted into acetaldehyde during the period of staining. Condition of staining, such as the pH of staining solution or the cofactor included in the staining solution, was not proper to stain soybean aldolase or other enzymes involved.

Amylase (AM). Three anodic bands with amylase activity have been observed (Gorman and Kiang, 1977). The first and the second bands are alphaamylase and third band is beta-amylase (Reiss, 1978; Hildebrand and Hymowitz, 1980). Four homozygous zymogram types were found in G. max and G. soja (Gorman and Kiang, 1977; Kiang et al., 1981; Kiang, 1981; Gorman et al., 1982b). Three of them, types 1, 2 and 4, were observed in this survey. Type 1 and type 2 differed in their mobility of the beta-amylase band and type 4 is a null type for that band (Fig. 3). It was suggested that the beta-amylase is controlled by a single locus with four alleles, Spl-a, Spl-b, Spl-an, Spl (Kiang, 1981; Kiang and Gorman, 1983; Palmer et al., 1985).

Acid Phosphatase (AP). Four homozygous zymogram types, differing in the mobility of the fastest migrating band (towards the anode) were found in G. max and G. soja (Gorman, 1983). All the four types (Fig. 4) were observed in this survey. Kiang and Gorman (1983) reported that several loci were responsible for encoding soybean acid phosphatase. The only genetically variable AP locus was found to have three codominant alleles, Ap-a, Ap-b, and Ap-c (Gorman, 1983; Hildebrand et al., 1980).

Diaphorase (DIA). Sixteen different homozygous zymogram types were found in G. \max and G. soja (Gorman, 1983), but only three of them, types 1, 2 and 9, were observed in this survey (Fig. 5). These three types may reflect the lower variations in cultivated soybeans than in wild soybeans. The difference between type 1 and 2 was at the bottom five bands (close to the origin). For type 2, only the lowest three bands were visible. Gorman (1983) hypothesized that this five-band cluster was the product of two DIA loci whose monomers interact to form intra- and interlocus tetramers. In zymogram type 9, the top two bands, bands 11 and 12, were missing.

Endopeptidase (ENP). Three homozygous ENP zymogram types were observed in this survey. They all had one single anodal band, but differed in their electrophoretic mobility. The Rf value of ENP band of types 1, 2 and 3 was 0.39, 0.40 and 0.43, respectively (Fig. 6). Genetic study revealed that type 1 and type 3 were controlled by a single locus Enp with two codominant alleles, Enp-a and Enp-b (Doong, 1986). Heterozygotes showed both parental bands without any intermediate band, indicating a monomeric structure for endopeptidase (Doong, 1986).

<u>Fluorescent Esterase (FLE)</u>. Two homozygous FLE zymogram types were found in this survey. One with five anodal bands was type 1; the other, lacking the first band, was type 2 (Fig. 7). Heterozygotes of these two types also showed five anodal bands, but the first band was with less intensity than that of type 1 (Doong, 1986).

Glutamate Oxaloacetic Transaminase (GOT). Three homozygous GOT zymogram types, all with four bands, were observed in cultivated and wild soybeans (Kiang et al., 1987). Two of the three types, which differed in the mobilities of the fourth band, were found in this survey (Fig. 8). Gorman (1983) hypothesized that these two zymogram types were caused by two variable alleles at a single locus.

NADP-active Isocitrate Dehydrogenase (IDH). Eight homozygous IDH zymogram types were observed in G. max and G. soja (Gorman, 1983; Kiang and Gorman, 1985). All eight types were observed in this survey (Fig. 9). Kiang and Gorman (1985) reported that the difference between zymogram types was the consequence of three unlinked loci with codominant alleles. Two of the four active IDH loci, coding for cytosol-associated IDH, interacted with each other to form intra- and inter-locus heterodimers (Gorman, 1983; Kiang and Gorman, 1985).

Leucine Aminopeptidase (LAP). Four homozygous LAP zymogram types were observed in G. max and G. soja (Gorman, 1983; Kiang and Gorman, 1983). Three of them, types 1, 2 and 4, were observed in this survey. Types 1 and 2 had a single anodal band, but differed in its mobility. The single band migrated faster in type 1 than in type 2 (Fig. 10). Kiang et al. (1985) reported that type 1 and type 2 variants were inherited as codominant alleles at the Lap1 locus. Type 4 was a null activity type at the Lap2 locus (Kiang and Chiang, 1987).

Malate Dehydrogenase (MDH). Only one MDH zymogram type was reported occurring in *G. max* and *G. soja*. No other variant type was found. A total of six bands was observed (Fig. 11). Bands 2, 3 and 4 were found to be associated with cytosol (Gorman, 1983; Kiang and Gorman, 1983).

Mannose-6-phosphate Isomerase (MPI). Four homozygous MPI zymogram types were observed in cultivated soybeans (Gorman et al., 1982b). They differed in their mobilities of the two MPI bands observed. An additional null type was found in wild soybeans (Gorman et al., 1983). Four of the five types, types 1, 2, 3 and 5, were observed in this survey (Fig. 12). Gorman et al. (1983) reported that a single locus with three codominant alleles, Mpi-a, Mpi-b, Mpi-c, was responsible for the difference between zymogram types 1, 2 and 3. Chiang (1985) confirmed that the fourth migrating variant was the product of a fourth codominant allele.

6-Phosphogluconate Dehydrogenase (PGD). Eight homozygous PGD zymogram types, with as many as six bands, were found in G. max and G. soja (Gorman, 1983). Three of them, types 1, 2 and 3, were observed in this survey (Fig. 13). Other types (4, 5, 6, 7 and 8) were very rare and had been observed only in wild soybeans (Gorman, 1983). The difference between types 1 and 2 was the mobility of band 1 (the closest band to the origin), which migrated faster in type 1 than in type 2. For type 3, band 1 was missing. Inheritance studies (Gorman et al., 1983) revealed that the first bands of these three types were the products of a single locus with variant alleles. It was also hypothesized that bands 1 and 3 were encoded by two interacting loci, and band 2 was the interlocus heterodimer (Gorman, 1983; Kiang and Gorman, 1983).

Phosphoglucose Isomerase (PGI). Four homozygous PGI zymogram types have been observed in soybean by Gorman (1983). Two additional types were found and were designated as types 5 and 6, respectively (Chiang, 1985; Doong, 1986). A three-band cluster was found at the location of band 1 in both types 5 and 6 instead of one band shown in the zymogram types 1 and 3 (Fig. 14). It was hypothesized that these three bands were encoded by two interacting loci which produced a homo-heterodimer complex (Chiang, 1985). Both types 1 and 3 had four anodal bands but differed in their mobility of the two fastest migrating bands. The zymogram differences between types 1 and 3

were suggested to be the consequence of a single nuclear locus with two variable alleles. This locus interacted with a second locus to form a fixed, three-banded homo-heterodimer complex (Gorman, 1983; Kiang and Gorman, 1983).

Phosphoglucomutase (PGM). Four homozygous PGM types were observed in the cultivated and wild soybeans (Gorman et al., 1982b; Gorman, 1983). Only two homozygous PGM zymogram types, 1 and 2, were found in this survey (Fig. 15). These two types differed in the mobility of the slowest migrating band towards the anode. This slowest band migrated faster in type 1 than in type 2. It was hypothesized that a single nuclear locus with two codominant alleles was responsible for the difference (Gorman, 1983).

Shikimate Dehydrogenase (SKD). Only one homozygous SKD zymogram type was found in this survey. This zymogram type, with three anodal bands, is shown in Fig. 16. No genetic data are available.

Soybean Trypsin Inhibitor (Ti). Three electrophoretic mobility variants and one null activity variant of Ti were observed. Inheritance studies revealed that the three mobility variants, Ti-a, Ti-b and Ti-c, were controlled by a single locus with three codominant alleles (Hymowitz and Hadley, 1972; Orf and Hymowitz, 1977, 1979; Hymowitz et al., 1978). All of the three types were observed in this survey (Fig. 17). The single Ti band of type a migrated faster than that of type b. Type c had the slowest migrating band.

Table 1. Zymogram types of named soybean cultivars

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Acadian	3	1	1	1	1	1	5	1	а
Acme	3	1	1	1	1,2	1	5	1	а
Ada	3	1	1	1	1	2	5	1	а
Adams	3	2	3	3	1	1	1	1	а
Adelphia	9	2	3	3	1	1	1	1	а
Agate	3	1	1	3	1	2	1	1	а
A.K. (FC 30.671)	4	1	1	3	1	1	1	1	а
A.K. (Harrow)	3	2	2	3	1	2	5	1	а
A.K. (Kansas)	6	1	1	3	1	1	5	1	а
Aksarben	3	1	1	3	1	1	1	1	а
Altona	3	1	1	1,3	1	2	1	1	а
Amcor	3	1	1	1	1	1	1	1	а
Amsoy	3	1	1	3	1	1	5	1	а
Amsoy 71	3	1	1	3	1	1	5	1	а
Anoka	4	1	1	3	1	1	5	1	а
Aoda	3	1	1	1	1	2	5	1	Ъ
Arisoy	3	1	1	1	1	1	5	1	а
Arksoy	3	1	1	3	1	2	5	1	а

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Amredo	3	1	1	3	1	2	1	1	a
Avayelles	3	1	1	1	1	1	5	1	a
Bansei	3	1	1	1	1	2	5	1	a
Bansei (Ames)	3	1	1	1	1	2	5	1	a
Barchet	3	1	1	1	1	1	5	1	a
Bavender Special A	3	1	1	3	1	1	1	1	a
Bavender Special B	3	1	1	3	1	1	1	1	a
Bavender Special C	3	1	1	3	1	1	1	1	a
Bedford	8	1	1	3	1	2	1	1	a
Beeson	8	1	1	3	1	2	5	1	a
Bethel	9	2	2	3	1	1	5	1	a
Biloxi	3	1	1	3	1	1	5	1	a
Black Eye	3	1	1	1	1	2	5	1	a
Black Eyebrow	3	1	1	3	1	2	5	1	a
Blackhawk	3	1	1	3	1	1	5	1	a
Bombay	3	1	1	3	1	1	1	1	a
Bonus	3	1	1	3	1	1	5	1	a
Boone	3	2	3	3	1	1	5	1	a
Bossier	3	1	1	3	1	1	1	1	a
Bragg	3	1	1	3	1	1	1	1	a
BSR 201	8	1	1	3	1	2	1	1	a
BSR 301	4	1	1	3	1	1	1	1	a
BSR 302	4	1	1	3	1	2	1	1	a
Burwell	3	1	1	1	1	2	5	1	b
Calland	3	1	1	3	1	1	1	1	a
Capital	3	1	1	3	1	2	1	1	a
Carlin	3	1	1	3	1	1	1	1	a
Cayuga	3	3	3	3	1	2	5	1	а
Centennial	3	1	1	3	1	1	5	1	а
Century	3	1	1	1	1	1	5	1	а
Charlee	3	1	1	3	1	2	5	1	a
Cherokee	3	1	1	3	1	1	1	1	a
Chestnut	3	1	1	1	1	1	5	1	a
Chico	3	1	1	3	1	2	1	1	a
Chief	3	1	1	3	1	2	1	1	a
Chippewa	8	1	1	3	1	1	5	1	a
Chippewa 64	8	1	1	3	1	1	5	1	a
Chusei	3	1	1	1	1	1	1	1	a
Clark	4	1	1	3	1	1	1	1	a

Table 1. Continued

Cultivar/enzymes	AC0	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Clark 63	8	1	1	3	1	1	1	1	a
Clay	3	1	1	3	1	1	1	1	a
Clemson	4	1	1	3	1	2	5	1	a
Cloud	3	1	1	1	1	2	5	1	a
CN 210 (2)	8	1	1	3	1	2	5	1	a
CN 290 (2)	8	1	1	3	1	1	5	1	a
Cobb	8	2	1	3	1	1	5	1	a
Coker 338	3	1	1	3	1	1	1	1	a
Coke Stewart	3	1	1	3	1	1	5	1	a
Coles	3	1	1	1	1	2	5	1	a
Columbia	3	1	1	3	1	2	5	1	a
Columbus	8	1	1	3	1	1	5	1	a
Comet	3	1	1	3	1	2	5	1	a
Corsoy	3	1	1	3	1	1	1	1	a
Corsoy 79	3	1	1	3	1	1	1	1	a
Crawford	3	1	1	3	1	2	1	1	a
Creole	4	1	1	1	1	2	5	1	a
Crest	3	1	1	1	1	2	5	1	a
Cumberland	4	1	1	3	1	1	1	1	a
Custer	4	1	1	3	1	2	1	1	a
Cutler 71	8	1	1	3	1	1	5	1	a
Cypress No. 1	4	1	1	3	1	2	1	1	а
Dare	3	1	1	3	1	2	5	1	а
Davis	3	3	3	3	1	2	5	1	а
Dawson	3	1	1	3	1	2	5	1	a
Delmar	8	2	2	3	1	1	1	1	a
Delsoy	3	2	2	3	1	1	5	1	a
Delta	3	1	1	3	1	1	5	1	a
Desoto	4	1	1	3	1	1	1	1	a
Disoy	3	1	1	1	1	2	1	1	a
Dixie	4	1	1	3	1	2	5	1	а
Dortchsoy 67	8	1	1	3	1	2	5	1	а
Douglas	3	1	1	3	1	1	1	1	а
Dunfield	3	1	1	3	1	1	1	1	a
Dunn	4	1	1	3	1	2	1	1	a
Dyer	3	1	1	3	1	1	5	1	a
Earlyana	4	1	1	3	1	2	1	1	a
Early White Eyebrow	3	1	1	3	1	2	5	1	a
Easy Cook	8	1	1	3	1	1	1	1	a

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Ebony Elf Elton	8 4 3	1 1 1	1 1 1	3. 3	1 1 1	1 1 2	1 5 1	1 1 1	a a a
Emerald	8	1	1	1	1	2	5	1	a
Emperor	3	1	1	1	1	2	1	1	a
Ennis I	4	1	1	3	1	2	1	1	a
Essex	9	3	3	3	1	2	5	1	a
Etum	3	1	1	1	1	2	5	1	a
Evans	3	1	1	3	1	2	5	1	a
Fabulin	8	2	2	3	1	2	5	1	a
Fayette	4	1	1	3	1	1	1	1	a
Flambeau	3	1	1	3	1	2	1	1	a
Ford	3	1	1	3	1	1	1	1	a
Forrest	3	1	1	3	1	2	5	1	a
Franklin	3	1	1	3	1	1	1	1	a
Fuji	3	1	1	1	1	1	5	, 1	a
Funk Delicious	3	1	1	1	1	2	5	, 1	a
Funman	3	1	1	3	1	1	5	1	a
Gaton	3	1	1	3	1	2	5	1	a
Georgian	3	1	1	1	1	2	5	1	a
Giant Green	3	1	1	3	1	2	5	1	a
Gibson	3	1	1	3	1	1	- 5	1	a
Gnome -	4	1	1	3	1	1	5	1	a
Goku	3	1	1	3	1	2	5	1	c
Goldsoy	3	1	1	3	1	2	5	1	a
Grand	3	1	1	3	1	2	5	1	a
Granger	4	1	1	3	1	2	5	1	a
Grant	4	3	3	3	1	2	5	1	a
Green and Black	3	3	3	3	1	1	5	1	a
Guelph	3	1	1	3	1	1	1	1	a
Harbaro	4	1	1	3	1	2	1	1	a
Harberlandt	3	1	1	3	1	2	5	1	a
Hakote	3	1	1	1	1	2	5	1	b
Hampton	4	1	1	3	1	1	1	1	a
Hampton 266A	3	1	1	3	1	1	1	1	a
Harbinsoy	3	1	1	3	1	1	5	1	a

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Harcor	3	1	1	3	1	2	1	1	a
Hardee	3	3	3	3	1	2	5	1	a
Hardin	3	1	1	3	1	2	1	1	a
Hardome	3	1	1	3	1	2	5	1	a
Hark	4	1	1	3	1	1	1	1	a
Harlon	3	1	1	3	1	1	5	1	a
Harly	3	2	2	3	1	2	5	1	a
Harosoy	3	1	1	3	1	1	5	1	a
Harosoy 63	3	1	1	3	1	1	5	1	a
Harman	3	1	1	3	1	2	1	1	а
Harrel	3	1	1	3	1	2	1	1	а
Harwood	3	1	1	3	1	1	1	1	а
Hawkeye	3	1	1	3	1	2	1	1	a
Hawkeye 63	3	1	1	3	1	2	1	1	a
Hayseed	3	1	1	1	1	2	5	1	a
Henn	3	1	1	3	1	1	5	1	a
Henry	3	1	1	3	1	2	1	1	a
Hidatsa	4	1	1	3	1	2	5	1	a
Higan	3	1	1	1	1	1	5	1	a
Hobbit	8	1	1	3	1	2	1	1	a
Hodgson	8	1	1	3	1	2	1	1	a
Hodgson 78	3	1	1	3	1	2	1	1	a
Hokkaido	4	1	1	1	1	2	5	1	a
Hollybrook	3	1	1	1	1	2	5	1	a
Hong Kong	3	1	1	3	1	2	5	1	a
Hood (6)	3	1	1	3	1	2	5	1	a
Hutton (8)	3	1	1	3	1	1	1	1	a
Hurrelbrink	3	1	1	1	1	2	1	1	a
Illington	3	1	1	1	1	2	1	1	a
Illini	3	1,2	2	3	1	2	1	1	a
Ilsoy	6	1	1	3	1	2	5	1	a
Imperial	3	1	1	3	1	2	1	1	a
Improved Pelican (8)	3	1	1	1	1	2	1	1	a
Jackson (8)	3	2	3	3	1	2	5	1	a
Jefferson	3	3	3	1	1	2	1	1	b
Jew 45 (8)	8	1	1	3	1	1	5	1	a

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Jogun	3	1	1	1	1	1	5	1	a
Jogun (Ames)	3	1	1	3	1	2	1	1	a
Jupiter	3	1	1	3	1	1	1	1	a
Kabott	3	1	1	3	1	2	5	1	a
Kagon	3	1	1	3	1	1	5	1	a
Kahala	8	1	1	3	1	2	5	1	a
Kaikoo	8	1	1	3	1	2	5	1	a
Kailua	8	2	2	3	1	2	5	1	a
Kanrich	3	1	1	1	1	2	5	1	a
Kanro	3	1	1	1	1	2	5	1	a
Knum	3	1	1	1	1	2	1	1	a
Kent	8	1	1	3	1	1	5	1	a
Kim	3	1	1	3	1	2	5	1	a
Kinston	3	1	1	3	1	1	1	1	a
Kingwa	8	1	1	3	1	2	5	1	a
Kino	8	1	1	3	1	2	1	1	а
Korean	3	1	1	3	1	2	5	1	а
Kura	3	1	1	1	1	2	5	1	а
La Green	3	1,2	1	3	1	1	5	1	a
Lakota	8	1	1	3	1	2	1	1	a
Laredo	3	1	1	1	1	2	5	1	a
Lawrence	3	1	1	3	1	1	1	1	а
Lee	3	1	1	3	1	1	1	1	а
Lee 68	3	1	1	3	1	1	1	1	а
Lee 74 Lincoln Lindarin	3 8 8	1 2 2	1 1 1	3 3 3	1 1 1	1 1 1		1 1 1	
Lindarin 63 Linman 533 Luthy	8 3 3	2 1 1	1 1 1	3 3 1	1 1 1	1 2 2		1 1 1	
Mack Macoupin Madison	8 3 3	1 1 2	1 1 1	3 3 3	1 1 1	2 2 1		1 1 1	
Magna Magnolia Majos	3 3 3	1 1 1	1 1 1	1 2 1	1 1 1	2 1 1		1 1 1	
Mamloxi Mammoth Yellow Mamredo	3 3 3	1 1 1	1 1 1	3 3 3	1 1 1	2 2 2		1 1 1	

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Manchu	4	1	1	3	1	2		1	
Manchu, Hudson	3	1	1	3	1	2		1	
Manchu, Lafayette	4	1	1	3	1	1		1	
Manchu, Lafayette 2	4	1	1	3	1	1		1	
Manchu, Madison	4	1	1	3	1	1		1	
Manchu, Montreal	3	3	3	3	1	2		1	
Manchu 3	4	1	1	3	1	2		1	
Manchu 606	4	1	1	3	1	2		1	
Manchu 2204	4	1	1	3	1	1		1	
Manchukota	3	1	1	3	1	1		1	
Manchuria	3	1	1	3	1	1		1	
Manchuria 13177	4	1	1	3	1	2		1	
Manchuria 20173	3	2	2	3	1	2		1	
Mandarin	3	1	1	3	1	1		1	
Mandarin, Ottawa	3	1	1	3	1	1		1	
Mandarin 507	3	1	1	3	1	1		1	
Mandell	4	2	2	3	1	1		1	
Manitoba Brown	3	2	2	1	1	1		1	
Manotan 664	3	2	2	3	1	1		1	
Mansoy	4	1	1	3	1	2		1	
Maple Amber	3	1	1	1	1	2		1	
Maple Arrow	3	1	1	1	1	2		1	
Maple Presto	3	1	ĩ	1	1	2		1	
Marion	3	1	1	3	1	1		1	
McCall	3	1	1	1	1	1		1	
Mead	8	1	1	3	1	1		1	
Medium Green	4	1	1	3	1	2		1	
Mendota	3	1	1	1	1	2		1	
Merit	3	1	1	3	1	2		1	
Midwest	3	3	3	3	1	1		1	
Miles	8	1	1	3	1	2		1	
Miller 67	3	1	1	3	1	1		1	
Mingo	3	1	1	3	1	1		1	
Minsoy	3	1	1	3	1	1		1	
Missoy	3	1	2	3	1	1		1	
Mokapu Summer	3	1	1	1	1	2		1	
Monroe	3	1	1	3	1	2		1	
Morsoy	3	1	1	1	1	2		1	
Mukden	3	1	1	3	1	2		1	

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Nanda	8	1	1	3	1	1		1	
Nansemond	3	1	1	3.	1	2		1	
Nebsoy	8	1	1	3	1	1		1	
Nela	8	3	3	1	1	2		1	
Norchief	3	1	1	3	1	2		1	
Norman (Amylase S)	3	1	1	1	1	1		1	
Norman (Amylase F)	3	1	1	1	1	2		1	
Noredo	8	1	1	3	2	2		1	
Norsoy	4	1	1	3	1	1		1	
OAC 211	4	1	1	3	1	1		1	
0akland	3	1	1	3	1	1		1	
Ogemaw	3	1	1	1	1	1		1	
Oksoy	8	1	1	3	1	2		1	
Old Dominion	8	1	1	3	1	2		1	
Ontario	4	3	3	3	1	2		1	
Otootan	3	1	1	3	1	1		1	
0saya	3	1	1	3	1	1		1	
Ozzie	3	1	1	3	1	2		. 1	
Pagoda	4	1	1	1	1	1		1	
Palmetto	8	1	1	3	1	1		1	
Pando	3	1	1	3	1	2		1	
Patoka	4	1	1	3	1	1		1	
Patterson	3	1	1	3	1	1		1	
Peking	8	1	1	3	1	2		1	
Peking 25s	8	1	1	3	1	2		1	
Pella	8	1	1	3	1	1		1	
Pennsoy	4	1	1	3	1	1		1	
Perry	4	1	1	3	1	1		1	
Pickett	3	1	1	3	1	1		1	
Pickett 71	3	1	1	3	1	2		1	
Pine Dell Perfection	8	1	1	3	1	2		1	
Pixie	8	1	1	3	1	1		1	
Platte	3	1	1	3	1	1		1	
Pluto	4	1	1	1	1	1		1	
Pochahontas	3	1	1	1	1	1		1	
Polland Yellow	3	1	1	3	1	1		1	
Pomona	8	1	1	3	1	1		1	
Portage	3	1	1	1	1	2		1	
Portugal	2	1	1	1	1	2		1	

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Prize Protana Provar	3 3 3	1 2 1	1 2 1	3 3 3	1 1 1	1 1 1		1 1 1	
Ralsoy Rampage Renville	3 8 3	1 1 2	1 1 3	3 3 3	1 1 1	2 1 2		1 1 1	
Richland Roanoke Rokusum	3 8 3	1 2 1	1 3 1	3 3 1	1 1 1	1 2 2		1 1 1	
Rose Non-pop Ross S-100	3 8 3	1 2 3	1 2 3	3 3 3	1 1 1	2 2 1		1 1 1	
Sac Sanga Sato	3 3 2	1 1 1	1 1 1	1 3 3	1 1 1	2 2 2	•	1 1 1	
Scioto Scott Seminole	4 8 3	1 1,2 1	1 1 1	3 3 3	1 1 1	2 2 2		1 1 1	
Semmes Shelby Shingto	1 8 4	1 1 1	1 1 1	3 3 3	1 1 1	2 2 2		1 1 1	
Shiro Simpson Sioux	3 3 3	1 1 1	1 1 1	3 3 3	1 1 1	2 2 2		1 1 1	
Sloan Sooty Sousei	3 8 3	1 1 1	1 1 1	3 3 3	1 1 1	2 1 2		1 1 1	
Soysota Sparks Sprite	8 3 8	1 1 1	1 1 1	1 3 3	1 1 1	2 2 2		1 1 1	
Steele Swift Tanner	3 3 3	1 2 1	1 1 1	3 3 3	1 1 1	1 2 1		1 1 1	
Tastee Tennessee Non Pop Toku	3 3 3	1 3 1	1 3 1	1 3 3	1 1 1	2 2 1		1 1 1	
Tokyo Tarheel Black Tortoise Egg	3 3 3	1 1 1	1 1 1	1 1 3	1 1 1	1 2 1		1 1 1	

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Tracy Traverse Union	8 3 8	1 1 1	1 1 1	3 3 3	1 1 1	1 1 1		1 1 1	
Vansoy Verde Vickery	3 3 3	2 1 1	3 1 1	3 1 3	1 2 1	1 2 2		1 1 1	
Viking Vinton Vinton 81	3 3 3	1 1 1	1 1 1	3 1 1	1 1 1	2 1 1		1 1 1	
Virginia Volstate Wabash °	8 8 3	1 3 1	1 3 1	3 3 3	1 1 1	2 2 2		1 1 1	
Ware Waseda Wayne	3 3 8	1 1 1	1 1 1	1 1 3	1 1 1	2 2 1		1 1 1	
Wea Weber Wells	3 3 8	1 1 1	1 1 1	3 3 3	1 1 1	1 2 1		1 1 1	
Wells II White Biloxi Wilkin	8 3 3	1 1 1	1 1 1	3 1 3	1 1 1	1 1 2		1 1 1	
Will Williams Williams 79	8 8 8	1 1 1	1 1 1	3 3 3	1 1 1	1 1 1		1 1 1	
Williams 82 Willomi Willomi B	8 3 3	1 1 1	1 1 1	3 1 1	1 1 1	1 2 2		1 1 1	
Wilson Wilson B Wilson 5	8 5 8	1	1 3 1	3 1 3	2 1 1	2 2 2		1 1 1	
Wilson 5B Wilson 6 Wing Jet	8 8 8	1 1 1	1 1 1	3 3 3	1 1 1	2 2 2		1 1 1	
Wirth Wisconsin Black Wolverine	8 4 2	1 1 1	1 1 1	3 3 1	1 1 1	1 1 2		1 1 1	
Woodworth Wood Yellow Wye	8 8 9	1 1 3	1 1 2	3 1 3	1 1 1	2 1 1		1 1 1	

Table 1. Continued

Cultivar/enzymes	ACO	ADH	ALD	ENP	LAP	FLE	PGI	SKD	Ti
Yellow Marvel	3	1	1	3	1	2		1	
Yelnanda	8	1	1	3	1	2		1	
Yelredo	3	1	1	1	1	2		1	
York	3	1	1	3	1	1		1	

Table 2. Zymogram types in the G. max germplasm

										1000	The Person of the Party of the					ĺ
T-strain/ enzymes	AC0	ADH	AM	AP	DIA	ENP	COT	IDH	LAP	MDH	MPI	PGD	PGI	PGM	SKD	Τi
T16 T31 T41	∞ m m		2 1 1	1 2 2	2 2 2	3 1 3		2 8 4			3 2 3			5 5 5		ט ט ט
T43 T48 T54	3 7 5	1 1 3	2 1 1	2 2 2	2 2 2	п п п		7 2 7		-i	2 2 2	1 1 1	2 2 2	2 2 2		ש ש ש
T93 T93A T102	e n n	2 1 1		7 7 7	7 7 7	m m m		8 2 1			2 2 2		1 1 2	2 2 2		ס ס ס
T104 T117 T122	3 4 5			222	1 1 2	3 3		6 4			2 2	1 1	1 1	2 1	1 1	ס ס
T134 T135 T136	6 W 4	2 2 1		7 7 7	2 2 1	3 3		6 5 5			7 7 7		1 1 1	. 7 7 1	1 1 1	ט ט ט
T138 T139 T143	m m m	1 2 1		5 5 5	7 7 7	еее		2 2			7 7 7		1 2	2 2 1		ט ט ט
T144 T145 T146	3 2 3	1 1 2		3 2 2	2 2 1	1 1 3	1 1 1	3 7			3 1 1	1 2 1	1 1 2	5 5 5		ט ט ט
T152 T153 T157	∞ ∞ m	2 2 1		2 2 2	1 2 2	m m m		9 1			7 7 7		1 22	2 2 2		ຫ ຫ ຫ

Table 2. Continued

Ţ	ש ש ש	р в в	т т т	0 0 0	рвр	т т т	авъ	n n n
SKD								
PGM	1 1 1	1 2 2	2 1 2	777	1 2 2	2 1 1	111	
PGI	2 22 2			1 1 1		5 1		1 2
PGD	1 1 1		2 1 1		1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7		1 1 3	
MPI	1 2 1	222	5 2 3	2 2 2	2 2 1	222	222	2 8 2
MDH								
LAP								
HQI	9 5 4	7 3 3	1 2 2	5 5 5	1 5 7	5 9 2	5 5	1 1
T05		1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	1 1 1	111
ENP	3 3 1	m m m	еее	ოოო	1 3 1	3 3 1	еее	еее
DIA	1 2 1	5 5 5	2 2 1		1 2 2	222	1 2 2 1	1 2 2
AP	7 7 7	5 5 5	1 2 2	5 5 5	7 7 7	5 5 5	2 2 1	2 2 2
AM	1 1 2						1 1 1	
АДН	1 2 1	1 2 2	1 1 2	5 5 5		2 2 2	1 2 2 1	2 1 1
AC0	m & m	986	∞ m ∞	∞ ∞ ∞	4 W W	m & v	4 6 6	3 0 8
T-strain/ enzymes	T160 T161 T162	T164 T171 T173	T175 T176 T180	T181 T201 T202	T204 T205 T208	T209 T210 T211H	T216 T218 T219H	T220 T221 T223

Table 2. Continued

T-strain/ enzymes	ACO	ADH	AM	AP	DIA	ENP	COT	IDH	LAP	MDH	MPI	PGD	PGI	PGM	SKD	Τi
T224 T225H T225M	m & &	1 1 2		222	1 5 5 7	m m m		1 6			222		1 2 2	1 1 2		ש ש ש
T226 T227 T229	6 8 4	1 2 2		2 2 2	1 2 2	тпп		- 2 2			5 5 5		1 1 2	2 2 1		ט ט ט
T230 T231 T232	m m m			1 2 2		ппп		W 10 10			222			1 2 1		в в в
T233 T234 T235	м∞м		1 1 2	2 2 2		епе		2 5 7			2 2 1		111	1 2 1		ט ט ט
T236 T238 T239	3 8 7		1 1 2	5 5 5	2 1 1	3 3 1		7 2 2			1 2 2	1 1 2	1 1 2	2 2 1		ຫຫຫ
T241H T242H T243	∞ m ∞	2 1 2		5 5 5	1 1 2	е е е		9 8 9			222		1 2 2	2 1 2		ט ט ט
T244 T249H T250H	4 & W	7 1 1	1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	222	2 1 1	ппп		5 2 7			2 7 1		7 1 1	1 2 1		ט ט ט
T251H T252 T253	ттт		2 2 2	5 5 5		m m m		~ ~ ~	4 1 1				1 5 5			ט ט ט

Table 2. Continued

T254 8 1 2 1 3 1 5 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 1 2 1 2 1 1 2	T-strain/ enzymes	ACO	ADH	AM	AP	DIA	ENP	COT	IDH	LAP	MDH	MPI	PGD	PGI	PGM	SKD	Τį
3 1 1 2 1 3 1 5 1 1 1 2 1 3 1 1 2 1 3 1 5 1	T254 T255	∞ ~			2.5		നന		70 50			2	1 -		2 -		יח ליח
3 1 2 1 3 1 5 1 1 2 1	T256	n en	-	-	2	1	. ες	П	2	-	-	-	-	-	2	-	to to
3 1 1 2 1 3 1 5 1	T257H	3	1	П	2	П	c	П	2	_	1	2	-	П	1	_	a
8 1 1 2 1 1 5 1 1 5 1	T258H T259H	ო ∞			2 2		m m		2 2			1 2			1 2		מ מ
3 1 2 2 2 1 3 1 3 1 1 1 1 1 5 1 1 1 1 1 1 1 1 1 1	Т260Н	œ	П	1	2	1	1	П	2	П	1	2	П	2	1	1	Ø
3 1 2 1 2 1 1 3 1 1 1 1 1 1 1 1 1 1 1 1	T261 T262	നന		2 2	2 2		ლ ო		7 3		1 1	1 2		2 2	1		вв
8 1 1 2 1 3 1 5 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1 1 2 1	T263 T264	m r		2	1 2		m «		7			1 0		- 5	1		as a
8 1 1 2 2 1 3 1 5 1 1 1 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1	Т265Н	0 00		П	2) M		2		П	7	1		2		ত ত
3 1 1 2 2 1 1 7 1	Т266Н Т267Н	∞ ~		1 %	2 6		m m		5			2			2 5		σπ
8 1 1 2 1 3 1 3 1 1 1 1 1 2 1 6 1 1 2 1 3 1 3 1 1 1 5 2 1 8 1 1 2 2 3 1 1 1 1 3 5 2 1 8 1 1 2 1 3 1 7 1 1 1 1 2 1	Т269Н	n m		۱ –	2	2	. –		7				1		ь п		क र
8 1 1 2 1 3 1 3 1 1 1 1 5 2 1 4 1 1 2 1 3 1 3 1 1 1 1 5 2 1 8 1 1 2 2 3 1 5 1 1 1 1 3 5 2 1 8 1 1 2 1 3 1 5 1 1 1 1 5 2 1 8 1 1 2 1 3 1 7 1 1 1 1 5 2 1	T270H	∞	П	П	2	1	3	1	3	П	П	1	1	-	2	1	В
8 1 1 2 2 3 1 5 1 1 1 3 5 2 1 8 8 1 1 2 1 3 1 7 1 1 1 1 1 1 5 2 1 1 1 1 1 1 1 1 5 2 1 1 1 1	T271H T272H	8 4			2		ოო		m m					5 5	7 7		מ מ
8 1 1 2 1 3 1 7 1 1 1 1 2 1	T273H T274H	∞ ∞	1 1		5 5	2	ო ო		5		1 1	1 1	3	2 2	7 7		מ מ
	T275	∞	П	1	2	1	3	1	7	1	П	1	1	1	2	П	В

Table 2. Continued

Τį	ט ט ט	ט ט ט	ט ט ט	фф	ט ט ט	ט ט ט	999	ט ט ט
SK								
PGM	222	2 1 2	2 2 2	222	7 7 1	2 5 1	2 2 2	7 7 7
PGI	2 2 2	5 5 5	1 2 2	5 5 9	1 5 5	1 1 2	1 1 2	1 22
PGD				1 1 2	222	2 2 1	1 1 2	1 1 2
MPI	222	2 1 2	7 7 7	1 1 2	2 1 1	1 1 2	1 2 2 2	2 2 2
MDH								
LAP								
IDH	50 00 00	2 3 7	9 8	988	∞ ∞ ∞	∞ ∞ ∞	∞ ∞ ∞	1 3 9
LOS								
FLE	222	2 1 2	2 2 2	7 1 1 7	1 1 1	2 1 2	222	2 1 2
ENP	ო ო ო	епе	ღ ღ ⊣	e e −	я – я	еее		3 3 7
DIA	7 7 7	2 1 2	1 2 1		1 2 1	7 1 5	E	7 7 7
AP	7 - 1 - 1	3 H	2 2 2	222	222	777	2 2 2	7 7 7
AM			1 1 1			7 7 7	1 2 1	
ADH			1 2 3					1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
ACO	α α α	m m m	7 8 9	4 9 6	m m m	m m m	m m m	m m m
FC-strain/ enzymes	01.547 02.208 02.109	03.548 03.609 03.654N	03.6541 04.002B 04.002N	04.007A 04.007B 19.976	19.9761 19.9762 19.9791	19.9792 19.9793 19.9794	19.9795 19.9796 19.9797	21.340 29.219 29.333

Table 2. Continued

FC-strain/ enzymes	AC0	АДН	AM	AP	DIA	ENP	FLE	COT	IDH	LAP	MDH	MPI	PGD	PGI	PGM	SKD	Ţ
30.233 30.683 30.684				000	1 5 5 7	m m m			m m m			1 2 1		1 5 5	1 2 1		ច្រ ប្រ
30.685 30.687 30.689	m m m			2 2 2	7 7 7	n n n	1 5 5		50 ∞ ∞			2 2 2	2 2 2	2020	1 1 1	~ ~ ~	ъаа
30.691 30.692 30.694	m m m	7 3 1		5 5 5		3 3 1			7 7 7					77 72 72	1 2 1		0 0 0
31,122 31,408 31,409	644			1 1 1	7 2 7	m m m	1 2 1		7 5 5			2 2 2		222	2 2 2	1	ф ф
31.557 31.571 31.5723	w w 4	- 8 -		2 2 1	2 2 2	3 3 1	1 1 2		1 7 2			1 2 2		2 2 2	2 2 2		рвв
31.579 31.630 31.678	3 6,8 4			222	2 2 2	നനന	1 2 1		7 1 5	1 2 1		1 H	1 1 2	1 2 1	2 7 1	1 1 1	0 0 0
31.684 31.685 31.697	4 6 6	7 7 7		222	7 1 5	ოოო	2 2 1		2 2 2	1		2 2 2			1 1 2		0 0 0
31.702 31.715 31.946	8 4 4	7 1 1		7 - 1 - 1	2 2 2	mmm	2 1 2		2 2 2			2 2 2		1 2	1 2 1		מ מ מ

Table 2. Continued

FC-strain/ enzymes	AC0	ADH	AM	AP	DIA E	ENP	FLE	COT	IDH	LAP	MOH	MPI	PGD	PGI	PGM	SKD	Ti
32.033 32.141 32.243	m m o	1 1 2	1 2 2	222	1 1 2	33.1	1 2 2		60 EV			H 1 2		1 5 5	7 1 7		w w w
PI No./ enzymes	AC0	ADH	AM	AP	DIA	1	ENP G		IDH	LAP	— — — WDH	MP.I	PGD	PGM		SKD	Ti
19.986 30.594 30.599	000			222	1 2 2		1 3 3		7 1 2			1 2 2	2 1 1	1 2 2	1 1 2		ששש
30.600 30.653 47.131	4 4 6		1 2 1	1 2 2	1 2 2				1 3 5			3 1 2		2 1 2	1 5 5		מ ש ש
54.583 54.591 54.592	8 4 8			5 5 5	7 7 7				3			232		2 1 2	1 1 2		~ ~ ~
54.6061 54.6062 54.607	7 4 4			2 2 2	2 2 1		m m m		1 2 3	111		3 2 2	2 1 1	2 1 2	222		8 8 8
54.608 54.6081 54.6082	α			2 1 1	2 1 1		3		7 7			2 3 2		7 7 7	1 2 1		w w w
54.6083 54.6084 54.6085	cn cn cn			1 2 2 7					L			7 7 7	1 2 2	1 2 1	1 1 2		8 8 8
54.609 54.6101 54.6104	3 7 10	1 2 2		7 7 7	7 7 7				7 1 1	1 - 1		1 2 2		1 1 1	1 1 2		ввы

Table 2. Continued

Ti	0 0 0	n n n	w w w	w w w	0 0 00	0 0 0	0 0 0	авъ
SKD		1 1 1	1 1 1			1 1 1		
PGI	5 1	1 5	5 2 1	1 1 2	5 1 1		5 5	1 2 2
PGM	2 1 2		2 1 1	5 5 5	2 2 2	1 2 1	2 2 2	2 1 1
I PGD	1 2 1 1	1 1 1			2 1 1	T T T	1 2 1 1	
MDH MPI	1 2 1 2 1 2	1 2 1	1 3	1 3	1 2 1 2 1 2	1 3 1 2 1 2	1 2 1 2	1 2 1 2
LAP M			1 1 4					1 2 1
IDH	N N N	1 7 1	N W N	3 3	5 1 5	7	3 3 7	3 1 1
COT	1 1 1			1 1 1	1 - 1	1 1 1	1 1 1	
ENP	3 1 3	m m m	m m m	നനന	3 3 1	m m m	m m m	m m m
DIA	2 1 1	1 2 2	2 2 2	1 1 2	2 2 2	1 1 2	2 2 2	2 2 2
AP	1 2 2	3 3 2	1 2 2	2 2 2	2 2 1 1	2 2 2	1 2 2 2	3 5 5
AM			1 2 1			1 2 1		1 2 2
АДН		1 2 1						
ACO	3,4 3	8 4 8	ммм	ене		നനന	7 4	4 8 8
PI No./ enzymes	54.615 54.6151 54.6152	54.617 54.618 54.620	54.6202 54.809 54.818	54.834 54.853 54.854	54.855 54.857 54.859	54.862 54.865 55.0891	55.873 55.887 56.563	57.334 58.955 59.849

Table 2. Continued

Ti	ששש	מ מ מ	ט ט ט	שמש	ט ט ט	рва	ט מ מ	ט ט ט
SKD								
PGI	1 1 1			5 1 5	5 5 5	N N N	1 1 5	1 2
PGM	1 2 2		2 2 2	2 2 2	2 2 2	2 2 1	1 2 2	1 2 2
PGD				1 2 2		7 - 1	7	
MPI	7 7 7	2 2 1	2 1 1	1 1 2	3 3	2 2 2	2 2 2	2 2 2
MDH								
LAP	1 4 1							
IDH	w w 10	3 / 2	3 22 22	0.00	3 3 7	m m m	2 2	. 2 1 2
LOĐ			1 1 2					
ENP	m m m	3 1 3	3 3 1	3 - 3	m m m	m m m	m m m	m m m
DIA	2 2 2	2 2 1	7 7 7	2 2	2 2 1	1 1 2	2 1 2	2 1 2
AP	2 2 2	1 2 2	1 2 2	2 2 2	2 1 1	3 2 2	2 2 2	2 2 2
AM								
ADH	1 2 2			1 3 1		3 1	5 2 3	2 1 2
ACO	еее	4 6 9	4 9 9	4 6 6	m m m		3 4 8	3 4 3
PI No./ enzymes	60.2692 60.272 60.279	60.2961 60.2962 60.970	61.940 61.944 61.947	62.199 62.202 62.2022	62.248 62.483 63.271	63.468 63.945 64.698	64.747 65.338 65.341	65.346 65.354 65.379

Table 2. Continued

Ti	ט ט ט	0 0 0	8 8 8	ю ю ю	ю <i>в</i>	в в в	в в в	0 0 0
SKD								
PGI	1 1 1	1 1 1			1 2			5 1 5
PGM	2 1 1	1 1 2	2 1 1	1 2 1	2 2 1	2 1 1	1 1 2	2 2 2
PGD	2 1 1		1 1 1					
MPI	777	2 1 2	282	2 2 1	888	1 2 2 2	2 2 1	1 2 2
MOH			1 1 1			7 7 7		
LAP		1 1 1			1 1 1	T T T		
HQI	1 1	2 3 7	5 7 5	200	5 7 5	7 5 5	2 2 2	7 5 1
GOT			1 1 1					1 1 1
ENP	3	п п п	е е е	ммм	n - n	ппп	п п п	ппп
DIA	222	2 1 2	1 2 2	1 1 1	1 1 2	5 2 3	1 2 1 1	2 1 1
AP	7 5 7	2 1 2	2 2 1	2 2 1	1 2 2	2 2 1	7 7 7	7 7 7
AM	1 1 4	1 1 1			1 2 1 1	2 1 1		
ADH	7 5 7	2 1 2	7 1 1	2 2 1	1 1 2	1 2 1	2 2 1	1 2 1
ACO	w m 4	949	4 4	. 44	4 4 4	7 7 7	7 7 7	3 4 8
PI No./ enzymes	65.388 68.011 68.398	68.410 68.421 68.423	68.427 68.430 68.436	68.439 68.443 68.446	68.448 68.449 68.454	68.455 68.457 68.4571	68.461 68.4611 68.465	68.4651 68.466 68.470

Table 2. Continued

Ti	ט ט ט ט	ט ט ט	ט ט ט	ט ט ט	נה נה נה	ט ט ט	ט ט ט	נה נה נה
SKD								
PGI	1 5 5	5 1 1	1 1 2		5 2 1		5 2 1	1 2
PGM	777	2 1 2	1 1 1	2 2 2	7 7 7	2 2 1	2 1 2	222
PGD								
MPI	7 3 1	5 2 3	3 2 2	7 1 7 5 7 5 7 1 1	2 1 2	2 2 1	2 5 1	2 2 2
MDH								
LAP								
HQI	იოი	1 5 7	2 2 2	2 2 3	. n n	2 2 2	5 7 1	5 7
COT								1 1 1
ENP	ттт	3 3 1	ппп	ппп	ппп	m m m	ппп	ппп
DIA	1 2 1	222	1 2 1		1 1 5	777	1 1 2	
AP	2 2 2	222	222	1 5 5 7	222	222	222	2 2 2
AM		~ ~ ~	2 1 1		2 1 1		7 - 1 - 1	
ADH	1 2 2	1 2 1	2 1 1	1 1 5	1 7 7	2 2 2		1 2 2
ACO	484	3 4 3	7 7 7 8	7 7 7	6 4 4	4 6 6	3 4 3	4 8 4
PI No./ enzymes	68.474 68.4742 68.475	68.4751 68.479 68.4791	68.480 68.481 68.483	68,4841 68,4844 68,488	68.494 68.500 68.503	68.508 68.516 68.521	68.5211 68.522 68.523	68.526 68.713 68.715

Table 2. Continued

T_1	ט ט ט	ט ט ט	ט ט ט	ט ט ט	ט ט ט	ט ט ט	ט א א	а ф а
SKD						1 1 1		
PGI	5 5	5 1 1			5 1	5 1	5 1 1	N N N
PGM	1 1 1	2 2 1	1 7 7	222	2 2 2	222	2 1 2	2 2 2
PGD								
MPI	2 1 2	222	2 2 2	7 5 7	3 3	3	1 2 2	1 2 2
MDH			1 1 1					
LAP								
IDH	1 1	E 2 2	5 7	3 5	3	5 5 5	5 P B	7 7
COT								
ENP	133	еее		m m m	133	ппп	ппп	m m m
DIA	2 2 1	2 1 2	777	1 2 1	1 2 1	7 7 7	1 2 1	2 5 1
AP	2 2 2	7 5 7	2 2 1	7 7 7	2 1 2	2 2 2	222	1 1 2
AM					1 1 1			
ADH	1 1 2	7 1 7	1 2 1	1 2 2	1 1 1	3 1 1	2 1 1 1	
ACO	7 7 7	6 9 4	7 7 8	7 6 7	e e 4	6 8 4	7 7 7	3 3 4
PI No./ enzymes	68.718 68.722 68.732	68.7321 68.736 68.741	68.746 68.748 68.7481	68.756 68.759 68.761	68.7613 68.762 68.763	68.765 68.768 68.770	68.778 68.788 68.795	68.806 68.815 69.500

Table 2. Continued

PI No./ enzymes	ACO	ADH	AM	AP	DIA	ENP	COT	IDH	LAP	MDH	MPI	PGD	PGM	PGI	SKD	Ti
69.501 69.503 69.507	4 6 4	2 7 1		2 2 1	2 2 2			222			222			1 1 1		משמ
69.5071 69.512 69.515	m m m		1 1 2	5 5 5	2 1 2	. 1 . 1		27 23		1 1 1	6 4 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6		2 1 2	5 1		0 0 0
69.532 69.533 69.991	e 4 e			5 5 5	2 1 1 1	е е е		1 2 2			222		1 2 2	1 1		ס ס ס
69.992 69.993 69.995	4 8 4			2 2 2	2 1 2	есе		N W N			3 2 5		7 5 7	1 1 2		0 0 0
69.996 70.001 70.009	998	222		2 2 2	7 1 1 7	m m m		5 7 5			5 5 5		1 1 2			о о о
70.013 70.014 70.016	4 4 6				2 2 1	നനന		1 2 2			1 2 2		2 7 1	1 2 1		р р р
70.017 70.019 70.021	777	1 5 2 5	1 2 2	222	2 2	m m m		5 2 7			1 2 2		2 1 1	5 1 1		ввы
70.023 70.027 70.036	m m m	1 1 2		5 2 4	2 2 2			. 2 - 2			3 3 1		1 2 1	1 2 1		0 0 0

Table 2. Continued

SKD Ti	1 1 a a a a	1	1	1 1 1 a a	1 1 1 a a	1 a a	1 I I a	1 I I
PGI	1 1 2	ν I ν	1 1 1	N N N		1 1 2	1 2	1 22 1
PGM	2 2 2	2 2 2	1 2 1		1 2 2	5 5 5	1 2 2	1 5 7
PGD				1 2 1			1 1 2	
MPI	23 2	3 3	2 2 2	2 2 2	2 3 2	2 2 1	2 1 2	2 1 2
MDH								
LAP			1 2 1					
IDH	3 1 1	200	5 7	1 7 3	5 7 5	2 2 2	1 2 7	2 2 7
LOS								
ENP		m m m	E 8 -1	m m m	е е е	m m m	3 3 7	m m n
DIA	5 5 5	2 1 1	2 2 2	2 2 2	2 2 2	5 5 5	1 2 1	1 5 7
AP	5 5 5	1 2 2	2 2 1	1 2 2	2 2 1	2 2 2	2 2 2	2 1 2
AM			1 1 1			1 2 1	7 7 7	
ADH	2 1 2		2 2 1		1 1 1		2 1 1	2
ACO	466	3 4 4	646	4 6 6	4 6 6	w w 4	7 8 7	4 60 6
PI No./ enzymes	70.076 70.077 70.078	70.080 70.084 70.087	70.089 70.091 70.188	70.189 70.192 70.197	70.199 70.201 70.202	70.208 70.212 70.213	70.224 70.228 70.229	70.241

Table 2. Continued

ACO	ADH	AM	AP	DIA		ENP	COT	HQI	LAP	MDH	MPI	PGD	PGM	PGI	SKD	Τί
4 6 6			1 2 2	222	0.000			7 5 5			7 1 7		222	5 5 1		ששש
8 7 7	2 2 1	1 1 2	2 1 2	2 7 2	(1(1)(1)			5 1 5			5 5 3		7 7 7	5 5		ט ט ט
ACO	ADH	AM	AP	DIA	ENP	FLE	COT	IDH	LAP	MOH	MPI	PGD	PGI	PGM	SKD	Τί
4 8 4	1 1 3		1 2 2	222	m m m	1 2		5 1 2			222		2 2 1		7 7 7	שמש
п п п	2 1 1			222	<u>е</u> е е е	1 1 5	1 1 1	5 5			222		1 5 1	2 2 1		ט ט ט
п п п	1 5 7 7	7 - 7	2 2 2	1 2 2	നനന	2 2 1		5 2			222					0 0 0
6 8 9	1 1 2		1 2 2	2 2 1	m m m			იოი			222	1 2 1		2 1 2		0 0 0
4 4	2		2	2 2	m m	2 2		\(\sigma\)			2		1	1 2		m m

Table 2. Continued

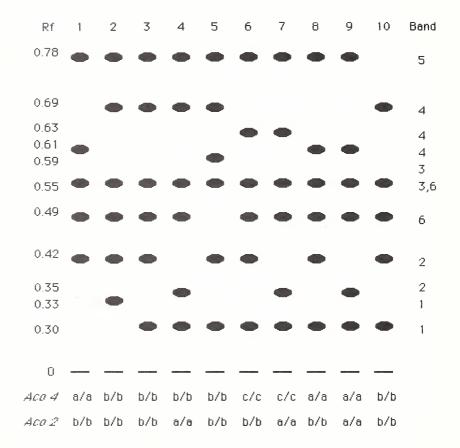
	AC0	ADH	AM	AP	DIA	ENP	FLE	COT	HQI	LAP	MDH	MPI	PGD	PGI	PGM	SKD	Ti
Cultivars from Korea																	
Backcheon Bongeui Hwang Keunkon	338	1 2 1		7 7 7	2 2 1	3 1	2 1 1		2 7 2			7 7 7	2 2 1		2 1 1		а Са
Jaeraejong	m	ı	_	2	П	3	2	1	∞	П	1	1	2	5	2	П	Р
333-8 Jang Yeobkong Kanglim	8 8	1 1	1 1	2 2	2	1 3	1 2	1	7	1 -		2 2	2		1 2	1 1	a vo
Kwangkyo KW26 S175040	m m m	2 1 1		2 2 2	5 5 5	3 3 1	7 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5 7 5	1 1 1	2 / 8	1 1 1		5 5 5	1 1 1	1 2 1	1 2 1		ввС
YN206 YN219 YN224	m m m		1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	2 2 2	2 2 2	m m m		1 1 1	5 2 7	1 1 1	1 1 1	5 5 5	2 2 1	1 1 2			ຫ ຫ ຫ
YN232 YN233-1 YN235	m m m		2 2 1	2 2 2	2 5 5	3 3 7		1 1 1	5 7 3	1 1 1	1 1 1	2 3 2	1 2 2	1 2	2 2 1		ט ט ט
74TC3-3-2 74TC4-6-1 74TC4-6-2	m m m		2 1 2	777	1 2 1	m m m		1 1 1	5 7 2		1 1 1	5 5 5			1 2 1		<i>v</i> v v
18022-2 22025-1	∞ m	п п		2 2	п п	п п	п п		7	T T	1	1 1	2	N N	2 2	1 1	ט ט

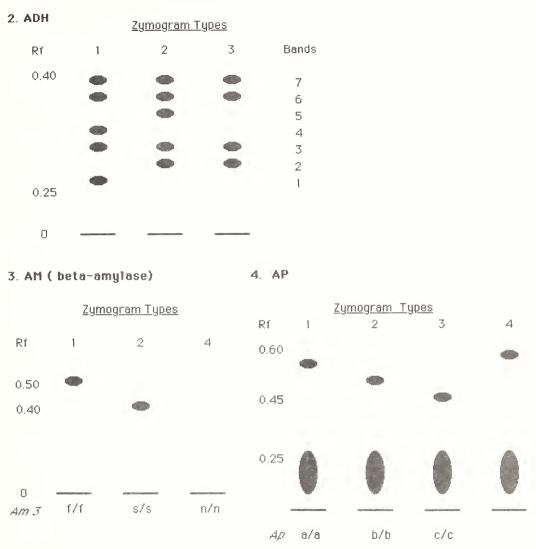
Table 2. Continued

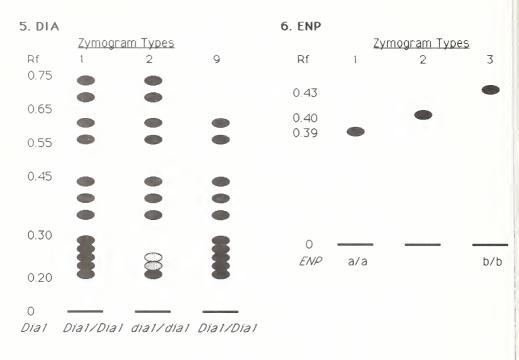
	AC0	АДН	AM	AP	DIA	ENP	FLE	COT	IDH	LAP	МДН	MPI	PGD	PGI	PGM	SKD	Ti
Cultivars from Taiwan																	l
AV38 AV57 AV62	m m ∞			2 2 2		3 3 1	1 1 2		2 2 2			1 1 2	1 2 2	1 2	1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	1 1 1	ש מ ש
AV66 AV68 AV69	6 4 5	2 1 2		3 2 2			2 2 2		7 4			1 1 2	1 2 1 1	1 1 2	1 2 2		т т т
AV70 AV73 AV215	m m m			232	2 1 1 1	m m m	2 1 1 1	1 1 1	7 2 3			1 2 2	2 2 2	1 1 2	2 2 2		а С а
AV2043 AV2120	2 3		1 1	3 2		3 -	1 2		4			1 2	1 2	5 5	1 2		т п

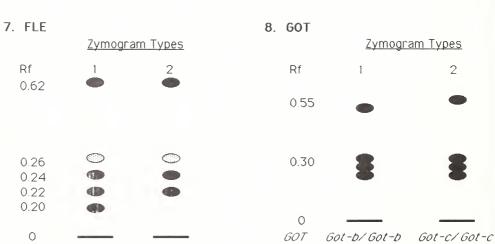
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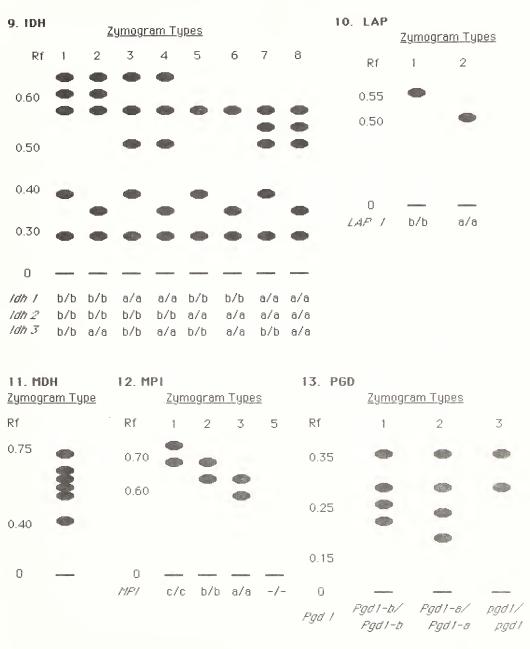
Zymogram Types

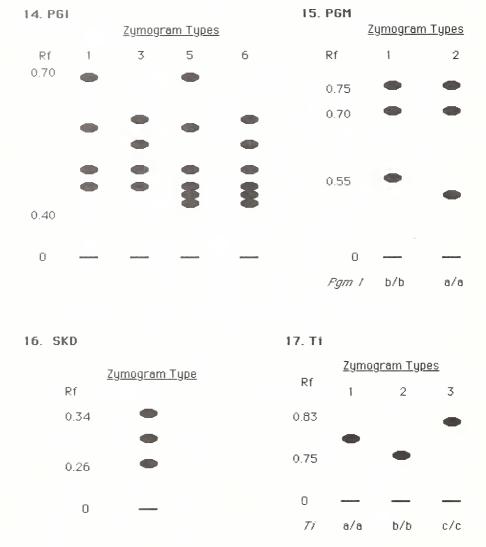












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Evaluation of soybean germplasm for stress tolerance and biological efficiency.

<u>Objectives</u>: To evaluate soybean germplasm and cultivars for stress tolerance toward moisture stress, pests, harvest index, micronutrients, diseases, nitrogen fixation and air pollution.

Moisture Stress - (B. Kpoghomou, V. T. Sapra and C. Beyl; Alabama A&M University, Alabama)

Three soybean cultivars, 'Lee-74', 'Wright', and 'RA 401', were subjected to 100% (control), 75%, and 50% of field capacity during vegetative (V1), flowering (R2), and pod-filling (R4) stages in greenhouse and field studies. Stress applied at R2 significantly reduced the yield in the greenhouse, while in the field, the maximum reduction was observed when the plants were subjected to stress at R4. Stress during V1 reduced the yield components less than stress applied during R2 or R4 stages in both studies. The pod number and seed weight were the yield components most affected by drought stress, and the number of seeds per pod the least affected. Yield stress index was significantly correlated with the yield and its components. The reproductive stage was clearly more sensitive to drought than the vegetative stage. The cultivar Lee 74 had the highest yield and the second highest yield stress index, whereas the cultivar RA 401 had the lowest yield (Table 1). Yield stress index was determined as: yield of stress treatments/yield of control plants x 100.

Pests - (M. Rangappa, M. E. Kramer and P. S. Benepal, Virginia State University, Virginia)

One of the major objectives of the proposal is systematic screening of all available soybean germplasm of Maturity Groups III to VIII for a natural resistance to a major insect pest, the Mexican bean beetle (MBB). These six Maturity Groups (III-VIII) cover about 5,000 plant introductions (PIs), 350 commercial varieties, and 80 breeding lines. Selected accessions of the most resistant (35% or less leaf defoliation) and highly susceptible (over 75% leaf defoliation) in general screening under field conditions were reevaluated in triplicate in 1985 and in quadruplicate in 1986 (Table 2). About 20 special selections were evaluated for MBB under controlled environmental conditions.

The research group at Virginia State University has developed a sophisticated MBB-rearing technique in the laboratory. Through this technique, each year an average of 25,000 to 30,000 adult MBB and different instar larvae are reared in the laboratory and released into the field experimental plots, thus creating high intensity levels of insect pressure to separate those accessions possessing high levels of natural resistance from those with low levels of resistance.

Besides the major thrust of screening for MBB, other secondary insect pests damage on soybean such as Japanese beetle, corn earworm, loopers, etc., air pollutants (03) sensitivity, virus symptoms and appropriate agronomic characteristics were recorded and incorporated into the data base for analysis.

An inducible defensive mechanism in soybean leaves, the trypsin inhibitors have been identified by using isoelectric focusing and gel electrophoresis in the process of working out mechanisms of resistance. Virginia State University takes pride in stating that this is the first such report in the literature of trypsin inhibitors in soybean leaves.

Harvest Index - (A. Bhagsari, Fort Valley State College, Georgia)

Eighteen soybean genotypes, six each from Maturity Groups (MG) V, VI, and VII were planted in four-row (6 m long and 0.9 m wide) experimental plots in a randomized complete block, with four replications, under field conditions using standard cultural practices. The objective was to determine seed yield efficiency (SYE), phytomass accumulation and leaf area index (LAI) development and other physiological and morphological traits and their relationships to yield. Significant differences were found in most of the parameters studied. The range in SYE was 45.2 to 52.1%, 46.5 to 54.7% and 43.9 to 48.3% for MGs V, VI and VII, respectively (Table 3). The new experimental lines, G81-152 (MG VI) had the highest SYE. Grain yield differed significantly within MGs and two genotypes ('Lefore' and 'G98-234') had grain yield of over 4 MT/ha. During September, phytomass yield varied from 6.6 MT/ha for 'Davis' to 12.3 MT/ha for 'Centennial' in MG VI. Generally, high-yielding genotypes also accumulated higher phytomass than lowyielding ones. During July, 60 days after planting, LAI varied from 1.6 to 3.2, 3.2 to 5.5 and 3.2 to 5.1 for MGs V, VI and VII, respectively. Within each MG, high yielding genotypes maintained higher LAI than the other genotypes near maturity.

Grain yield was significantly correlated (r = +0.74 - 0.83) to LAI, phytomass (r = +0.78 - 0.96) and number of pod-bearing branches (r = +0.79). Highly significant correlation coefficients were also found between LAI and total phytomass (Table 4). Within each MG, grain yield was significantly correlated with phytomass. Seed yield efficiency showed no significant correlation.

(J. Joshi and R. Padson, University of Maryland, Maryland)

Selected cultivars from Maturity Groups III, IV, and V were evaluated for the relationship between harvest index (H.I.) and other plant traits. Six PIs with high H.I., six with low H.I. and one adapted variety were included in the experiment from each of the three Maturity Groups.

Previous data (1985) had shown a wide range of H.I. in each Maturity Group. Harvest index ranged from 0.39 to 1.36 from 0.41 to 1.38 in MG IV and from 0.35 to 1.52 in MG V.

 $Plant\ height,\ yield,\ and\ yield\ stress\ index\ of\ three\ soybean\ cultivars\ under\ drought\ stress\ during \ VI,\ R2,\ and\ R4\ stages\ in\ the\ greenhouse$ Table 1.

		25% c	25% of field capacity	city	20%	50% of field capacity	Ĺty
Cultivar	Control	Vl	R2	R4	V1	R2	R4
				Plant height (cm)			
Lee 74	28.89 b ⁺	18.33 b	18.33 b	27.33 ab	15.33 a	28.33 a	29.33 b
Wright	30.63 a	21.17 a	29.83 a	31.16 a	17.70 a	31.17 a	32.83 a
RA 401	26.22 b	17.50 b	26.00 b	25.50 b	14.66 a	24.50 b	24.10 c
				Yield plant ⁻¹ (g)			
Lee 74	27.23	19.22 b	13.70 a	15.48 b	15.91 a	7.75 a	10.12 b
Wright	30.23	20.85 a	14.51 a	17.75 a	18.33 a	5.64 b	13.71 a
RA 401	22.29	17.72 b	11.82 a	13.85 b	12.26 a	7.63 ab	9.30 b
			Y	Yield stress index ((%)		
Lee 74		67.03 b	52.22 a	56.67 a	55.40 a	29.59 a	37.12 b
Wright		68.18 b	45.97 a	62.58 a	60.00 a	17.90 b	48.33 b
RA 401		82.43 a	50.79 a	63.20 a	57.30 a	32.98 a	42.81 b

*Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's Multiple Range Test.

Table 2. Total soybean germplasm screened for Mexican beam beetle in the field and final selections

		Screened germplasm	ermplasm		Selections	ctions
Maturity groups	PIs	Cultivar	Breeding lines	Total	Most resistant	Highly susceptible
III	1087	38	3	1128	17	15
ΔI	2240	52	6	2301	10	5
Λ	1369	43	12	1424	65	12
ΙΛ	421	33	15	697	45	15
VII	314	28	∞	340	34	11
VIII	266	12	9	284	13	10
Total	7697	206	53	5946		89

Seed yield efficiency, grain yield, phytomass, and number of pod-bearing branches in soybeans (1986) Table 3.

	Seed	\$ 0 0 0			-Phytomass			Pod-bearing	ring
	yieiu efficiency	yield	9 Oct	25 Sept	4 Sept	18 Aug	21 July	branches	nodes
	%		-	TM	MT/ha			Number/plant	lant
Group V									
417.172	52.10	2.23	4.28	4.26	4.97	5.27	1.77	3.16	8.00
416.838	51.89	2.32	4.42	3.45	4.28	4.98	2.06	3.00	8.41
416.447	47.66	1.92	40.4	6.87	5.13	3.63	2.10	3.66	9.42
Essex	46.00	2.75	5.99	4.27	6.44	3.86	2.40	4.42	9.00
417.356	45.90	2.73	5.97	4.56	4.57	4.77	2.66	99.4	9.00
417.493	45.17	1.88	4.12	4.97	7.01	3.76	2.39	4.33	8.17
Group VI									
G81-152	54.70	3.74	6.85	10.16	9.34	7.11	4.29	5,33	8.25
Davis	54.17	2.98	5.51	6.63	6.62	4.62	2.88	3.00	10.50
G98-234	49.29	4.05	8.27	10.09	7.91	5.47	3.22	6.41	8.08
Tracy	49.09	2.90	5.97	8.72	5.23	5.20	2.41	4.75	9.58
Centennial	46.68	3.73	7.98	12.31	9.01	5.83	3.00	6.75	10.50
Leflore	46.54	4.12	8.83	11.49	5.94	7.31	3.54	5.75	9.41
Group VII									
G80-1011	48.28	3.06	6.32	8.00	4.97	4.04	2.17	5.25	9.33
Ransom	47.64	3.13	6.59	5.59	5.60	4.47	2.60	4.79	8.54
Braxton	47.29	3.18	6.73	6.16	4.20	3.00	2.18	5.92	10.75
G80-1413	46.41	3.89	8.34	11.67	7.66	7.68	3.20	6.16	9.18
Wright	46.07	3.01	6.75	7.64	5.74	5.56	2.62	4.42	9.17
Gordon	43.87	3.54	8.07	10.23	5.77	7.36	3.96	6.91	10.16
L.S.D. (0.05)	4.83	0.51	0.92	2.79	2.88	1.42	1.37	1.97	1.26
(within groups	ps)								

Table 4. Correlation coefficients (γ) for soybeans (1986)

	Grain yield	Total phytomass	
^z LAI Oct 9	+0.42 ^{N.S.}	+0.51*	
LAI Sept 25	+0.83**	+0.86**	
LAI Sept 4	+0.80**	+0.75**	
LAI Aug 18	+0.80**	+0.79**	
JAI July 21	+0.74**	+0.70*	
No. pod bearing branch	+0.79**	+0.86**	

ZLAI (Leaf Area Index).

Micronutrients - (M. R. Reddy, North Carolina A&T State University, North Carolina)

A greenhouse study was conducted to evaluate the sensitivity and tolerance of various soybean genotypes to high levels of soil manganese, and to strongly acid soil conditions. Forty-four soybean genotypes in Maturity Groups V, VI, VII, and VIII were evaluated, the soil used for the study was very high in manganese (90 ppm) and strongly acidic (pH 4.8), and was collected from Buncombe County, North Carolina. The soil pH levels were pH 4.8 (original soil pH), and pH 6.3.

Genotypes PI 200506, FC 31737, FC 417136, PI 123440, PI 89469, L-76-0049, 'Coker 237', and 'Bedford' were sensitive to high levels of manganese and low soil pH; seed yield decreased significantly at pH 4.8. These genotypes did well at pH 6.3 and gave significantly higher seed yield. Genotypes PI 381668, FC 31665, PI 417063, PI 416900, PI 960895, PI 159319, PI 170891, PI 181565, and some others were not sensitive to high manganese and low soil pH, and their seed yield was not significantly different under the different soil pH levels (Table 5). Genotypes PI 200474, PI 229358, PI 416893, PI 416937, PI 417134, and 'Deltapine' were tolerant to high soil manganese and to low soil pH, but did not do well at higher soil pH (pH 6.3), resulting in lower seed yield (Table 6). The genotypes that were sensitive to high soil manganese showed higher concentration of manganese in leaf tissue compared with the tolerant genotypes (Table 7).

Diseases - (R. P. Pacumbaba and V. T. Sapra, Alabama A&M University, Alabama)

A field study was conducted for screening and selection of improved soybean germplasm for disease resistance in 1986. The soybean crossing block consisted of 207 germplasm entries screened at flowering and at maturity. One hundred and one were resistant and moderately resistant to bacterial blight (BB). Thirty soybean germplasm entries supplied by Auburn University

^{*,**}Significant at 5% and 1% levels of probability, respectively.

N.S. = Not significant.

Table 5. Soybean genotypes tolerant to high soil manganese and low soil pH, with similar yield under different soil conditions

Soybean genotype	Soil pH 4.8 (high soil Mn)	Soil pH 6.3 (low soil Mn)
	Seed yield	(g/pot)
PI 96089	5.0	5.5
PI 159319	11.1	12.4
PI 159322	6.2	7.1
PI 170891	8.5	7.9
PI 171442	5.3	5.6
PI 181565	9.7	8.9
PI 230978	6.8	6.7
PI 279621	7.3	7.1
PI 324068	6.5	5.5
PI 379618	6.8	7.1
PI 381668	8.3	9.3
PI 416900	7.4	7.1
PI 417063	8.1	8.0
PI 417123	5.8	6.4
PI 417258	6.5	7.2
PI 423824	5.6	5.4
PI 423986	5.6	6.2
PI 960895	7.1	8.6
FC 31665	7.2	8.6
L-76-0132	7.2	5.7
Easy Cook	6.7	6.7

Table 6. Soybean genotypes tolerant to high soil manganese and low soil pH, with lower yield at higher pH $\,$

Soybean genotype	Soil pH 4.8 (high soil Mn)	Soil pH 6.3 (low soil Mn)
	Seed yiel	d (g/pot) ———
PI 200474	8.4	6.5
PI 229358	7.6	5.1
PI 416893	10.2	8.1
PI 416937	9.7	5.7
PI 417134	9.4	7.2
Deltapine	8.8	6.1

Table 7. Differential uptake of manganese by various soybean genotypes

Soybean genotype	Soil pH 4.8 (high soil Mn)	Soil pH 6.3 (low soil Mn)
Consideration to Ma	Leaf Mn	(µg/g) ———
Sensitive to Mn		
PI 200506	437	200
L-76-0049	282	190
Bedford	277	198
Tolerant to Mn		
PI 159319	247	192
PI 170891	255	189

for breeding purposes were also screened for disease resistance. Twenty-four were resistant to soybean stem canker (SSC), two and ten were resistant and moderately resistant to BB. Rating for soybean cyst nematode (SCN) was not included because the soybean was planted in non-SCN infested soil. In the Uniform Soybean Test, 13 germplasm entries (selected from an earlier study) were used. Six and 13 germplasm entries were moderately resistant to SCN and BB, respectively, while 12 and one entries were resistant and moderately resistant to SSC. In the soybean collection of the RR3 project, 58 entries were screened for resistance to SSC and BB. Forty-one entries were found resistant to SSC and one and eight were resistant and moderately resistant to BB. Five germplasm entries showed multiple disease resistance ('Laneer', 'Jeff', 'AM 1026', PI 157476, and 606).

Nitrogen Fixation - (M. Floyd and S. Mookherji, Alabama A&M University, Alabama)

Sixty-five soybean germplasm lines from maturity groups (MG) IV to VIII were evaluated for their acid (pH 4.4) and Al (6 ppm) tolerances by measuring their N $_2$ fixing capabilities with USDA rhizobium strain 110 and mixture of strains 110, 6, and 122. Plants were grown in a growth chamber in nitrogen-free nutrient solution for 35 days. Plant introductions from MG VI had consistently higher (\geq 100%) relative root length in 6 ppm Al nutrient solution than those lines from other MGs. Germplasm lines within MG VII had the highest nodule numbers (16.5) per plant (Fig. 1) and also the highest nitrogenase activity (4.68 µmole $\rm C_2H_4/plant/hour)$ (Table 8). A comparison of the means of N-fixing traits showed that germplasm lines within MG VII performed better than lines from MG V and VI at pH 4.4 (Table 9). Germplasm 'Deltapine 246', 'Deltapine 560', and PI 416893 had the best symbiotic response.

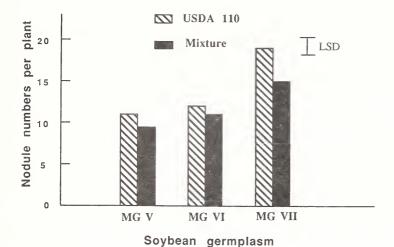


Fig.1 Effect of Rhizobia Strains on Nodule Numbers

Table 8. Comparisons of means of N-fixing traits of soybean germplasm lines from MG V-VII at pH 4.4 $\,$

Soybean germplasm	Nodule no. per plant	ARA μ mole C ₂ H ₄ /hr/plant	Total % N in shoot
MG V	9.95 b**	2.86 b	3.42 a
MG VI	11.59 b	3.45 b	3.80 a
MG VII	16.47 a	4.68 a	4.00 a

^{**}Numbers followed by the same letters within columns are not significantly different at p = 0.05 by DMRT.

Table 9. Symbiotic performance of soybean germplasm lines from MG VII at pH 4.4 inoculated with USDA 110

Soybean germplasm lines	Nodule number per plant	ARA µmole C ₂ H ₄ /hr/plant	Total % N
Deltapine 246	14.53 bc**	3.59 bc	4.47 a
Deltapine 560 PI 416893	20.70 ab 22.33 a	4.34 ab 8.12 a	4.40 a 3.57 b

**Means followed by the same letter are not significantly different at probability = 0.05 by DMRT.

Air Pollution - (G. Gupta and R. B. Dadson, University of Maryland, Maryland)

Relationships between the three major ambient air pollutants - ozone (0_3) , sulphur dioxide (SO_2) and nitrogen dioxide (NO_2) - have been explored experimentally using linear regression analysis. It has been shown that the amount of 0_3 in ambient air is based on the total amount of $\mathrm{SO}_2+\mathrm{NO}_2$ and that the concentration of 0_3 can be predicted from the concentration of $\mathrm{SO}_2+\mathrm{NO}_2$. On the basis of the data collected, a modified NO_2-0_3 photolytic cycle has been proposed taking into account dry precipitation of SO_2 and NO_2 .

One-month-old soybean cultivar 'Williams' was exposed to NO2 at 0.5 and 0.1 ppm concentration in growth chambers for only 7 hours. Data were collected both on net photosynthetic rate (before exposure, immediately following exposure and 24 hours after exposure) and yield (pod number, seed number and dry seed weight per plant). Net photosynthetic rates immediately following exposure to 0.5 and 1.0 ppm NO2 treatment decreased by 18% and 27%, respectively, compared with the control plants exposed to carbon filtered air. Reductions in yield were observed with both the 0.5 and 1.0 ppm NO2 treatments. The reduction in yield was linearly related to NO2 concentration; with 0.5 and 1.0 ppm NO2, the yield (seed weight per plant) reduction was 35 and 60%, respectively. Waller Duncan - K ratio t test and the Tukey's studentized range (LSD and MSD) tests showed that the treatments were significantly different at 0.05 level both for reduction in photosynthesis immediately following exposure and yield data.

The study on the effects of 0.0, 0.1, 0.2, 0.3, and 0.5 ppm levels of ${\rm NO}_2$ exposure on soybean cultivar Williams in relation to photosynthesis, protein content, chlorophyll content, membrane permeability, biomass, and yield data is in progress. Other cultivars also will be studied with the long-range objective of learning the combined effects of ${\rm O}_3$, ${\rm SO}_2$ and ${\rm NO}_2$.

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 An improved greenhouse method of evaluation for inheritance of resistance to race 4 of soybean cyst nematode.

Studies to determine mode of inheritance of resistance to soybean cyst nematode (SCN), Heterodera glycines, could be influenced by the methods of evaluation in use. Any improvement in growing conditions of the host and pathogen, superior inoculation techniques, inducement of desirable infection in the host with minimum genetic variability in the SCN population could result in optimum expression of full complement of genes conferring resistance to SCN races.

Our objective was to determine the mode of inheritance of resistance in Soybean Plant Introduction PI 88.788 for SCN race 4 reaction, using the improved techniques of evaluation.

Crosses were made in the field during summer between resistant parent PI 88.788 and susceptible parents 'Peking' and 'Forrest'. The F_1 and F_2 plants were grown in Puerto Rico and University of Missouri-Columbia Delta Center, Portageville, to obtain F_2 and F_3 seeds, respectively. Care was taken to grow plants in fields without SCN infestation.

The F_2 plants, their parents, and a set of standard host differentials, with 'Essex' as susceptible check, were evaluated in the greenhouse. The basic techniques of evaluation and methods for preparation of purified SCN race 4 inoculum were the same as used by Anand and Brar (1983) and Rao-Arelli and Anand (1986).

Some of the improvements include: (1) selection, reproduction of SCN race 4 field populations on susceptible PI line 90.763 for more than 30 generations under isolation in the greenhouse to minimize the existing genetic variability; (2) preparation of inoculum from freshly picked white females found on the roots of PI 90.763 and crushed to release eggs for obtaining synchronized hatching; (3) using inoculum entirely consisting of eggs and placing in direct contact with the host roots for quicker hatching and efficient utilization of all available infestation sites; (4) an aquarium airblowing pump was used to keep eggs constantly in uniform suspension, so as to dispense approximately same number of eggs in each inoculation.

The reaction of the plants for race 4 is presented in Table 1. The Index of Parasitism (IP) was calculated [(number of cysts on a given PI line/number of cysts on susceptible, Essex) \times 100]. The reaction was expressed positive or susceptible where IP was 10% or more (Golden et al., 1970).

Of the 200 $\rm F_2$ plants tested from the cross Forrest x PI 88.788, 25 were resistant and 175 were susceptible. The cross of Peking and PI 88.788 segregated 22 resistant:177 susceptible in $\rm F_2$. The segregation in both crosses could be explained based on two dominant genes and one recessive gene in PI 88.788 conditioning resistance to SCN race 4.

Table 1. Reaction of soybean parents, F2 plants, host differentials and Essex to Heterodera glycines race 4

	Number o	f plants	Expected	χ ²	Р
Entry	Resistant S	Susceptible	ratio	value	value
Peking	0	10			
Forrest	0	10			
PI 90.763	0	10			
PI 88.788	10	0			
Essex	0	10			
Forest x PI 88.788 (F ₂ s)	25	175	9(R):55(S)	0.36	.5070
Peking x PI 88.788 (F ₂ s)	22	177	9(R):55(S)	1.47	.2030

Several genes conditioning resistance to SCN races were reported in soybean lines Peking, PI 90.763 and PI 88.788 by Caldwell et al. (1960), Hancock et al. (1985), Hartwig and Epps (1970), Matson and Williams (1965) and Sugiyama and Katsumi (1966). Thomas et al. (1975) indicated that resistance to SCN race 4 was conferred by a single recessive gene pair in the cross PI 88.788 \times Peking.

The F_2 results obtained in our studies appear to indicate a more complex nature of inheritance for controlling SCN race 4 resistance in PI 88.788. Presumably, the expression of full complement of genes to SCN race 4 in PI 88.788 occurred with the use of some of the improved techniques of evaluation available today. Of course, evaluation of F_3 families would confirm the results obtained in F_2 generations.

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1) Response of soybean strains to DPX-F6025 in hydroponics.

Introduction: Previous work with DPX-F6025 (2-(([(4-chloro-6-methoxy-pyrimidine-2-yl) amino carbonyl] amino sulfonyl)) benzoic acid, ethyl ester) found differential strain response to increasing rates in hydroponics (Lloyd, 1985). Hanson (1984) reported differential strain response to a single rate of metribuzin in soybeans when evaluated in a hydroponic system similar to one developed by Barrentine et al. (1976).

With known agronomic changes in soybeans associated with herbicide treatments, this study was undertaken to study the agronomic effects of DPX-F6025 on a randomly selected group of soybean strains when evaluated in a hydroponic system.

<u>Materials and methods</u>: This experiment was conducted in the greenhouse in the winter of 1984-1985. Initially, 86 randomly selected cultivars, experimental lines and plant introductions were selected for evaluation, but the number of strains was reduced to 72 after the initial evaluation to reduce the space required in the greenhouse.

Greenhouse evaluation was conducted in a hydroponic system by first germinating 20 seeds of each strain in 10 cm sand-filled plastic pots at a depth of approximately 4 cm, 10 seeds per pot. One week after planting, or when the plants were in the early crook stage, the plants were removed from the pots, the sand was washed from the roots and 5 plants per strain were placed in a row through 7.5 mm holes drilled through 1.7 cm thick open-cell white styrofoam sheets measuring 26.7 by 30.5 cm. Four strains were placed in each sheet giving a total of 20 plants per sheet. Plants were 5 cm apart, equidistant in all directions.

The styrofoam sheets were then floated on 8 L of 1/2X modified Hoagland's solution in brown plastic dishpans measuring 29.2 by 34.3 by 13.4 cm (Hoagland and Arnon, 1950; Crafts-Brander and Harper, 1982). The solution was not aerated and no pH control was provided. The pH was checked at the beginning of the experiment and again at the end with no appreciable change from a value of 7.4. Metalaxyl, trade name Ridomil®, at 0.2 ml L $^{-1}$ was added to control the incidence of phytophthora in the system.

The experimental design was a randomized complete block design with a factorial arrangement of strains and rates with two replications. Two rates of DPX-F6025 were used, 0 and 2 mg L^{-1} a.i. in hydroponics. Approximately 7 days after transplanting into the styrofoam sheets or when the unifoliolate leaves had unfolded, the level of solution was checked and brought back up to 8 L. An aliquot of DPX-F6025 containing 64 mg of product in 5 ml of water was added to the containers receiving the 2 mg L^{-1} treatment and stirred, those pans receiving no treatment were also stirred.

After approximately 10 days, the plants were harvested by clipping the stems immediately above the cotyledons and readings on plant height in cm and fresh weight in gm were taken on the group of 5 plants. In addition, a

rating was taken on red venation based on a scale of 0 to 100 with 0 being no reddening, 100 reddening to the tip of the leaf. A general rating based on general plant appearance also was taken, again on a scale of 0 to 100, with 0 being mortality, 100 being no injury.

Results and discussion: Plant height, fresh weight and general rating were all significantly lower as the rate of DPX-F6025 increased, but exhibited no significant changes due to strain and no significant rate-by-strain interaction. Red rating was significantly greater as the rate of DPX-F6025 increased and did show a significant change due to strain and a significant rate-by-strain interaction. This indicated that, of the variables measured, red rating gave the best indication of the level of injury of individual strains evaluated.

When the variables were analyzed within rates, no significant strain effect was found for any of the variables at the 0 mg $\rm L^{-1}$ rate. Significant differences due to strain were found for plant height (alpha = 0.10), red rating, and general rating at the 2 mg $\rm L^{-1}$ rate. All the variables, plant height, red rating, and general rating, were useful in distinguishing sensitivity of lines to DPX-F6025.

Based upon the red rating obtained from evaluating in 2 mg $\rm L^{-1}$ a.i. DPX-F6025, the strains were ranked from those showing a high red rating to those showing a low red rating (Table 1). There was no attempt to try to classify the strains into sensitive, intermediate, or tolerant classes. The high value for the least significant difference and the continuous gradation of ratings between those strains showing high levels of injury and those with low levels of injury would have made any attempts at classification arbitrary. Some strains exhibited high levels of tolerance with few escapes. Other strains showed a high degree of sensitivity with no tolerant plants. Individual comparison could be made between selected strains by the use of the least significant difference.

Conclusion: Differences in the amount of injury occurred in different soybean strains, indicating levels of sensitivity to DPX-F6025. Plant height, fresh weight, general rating and red rating were all significantly affected by rate of DPX-F6025, but only red rating showed a significant strain effect and a significant strain-by-rate interaction. This indicated that red rating was the most reliable predictor of how an individual strain would react in hydroponics. The strains could be ranked according to their red rating scores, but no reaction classes could be detected.

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Table 1. Average red rating scores for soybean strains treated with 2 mg $\rm L^{-1}a.i.$ DPX-F6025 in hydroponics

Strain	Rating ⁺	Strain	Rating
LN81-1029	95	HW79149	40
LN81-1044	95	A78-123018	35
U80-64032	95	M74-62	35
C1631	90	LN80-9729	35
HW8221	90	Corsoy 79	35
Essex	90	Zane	35
Century 84	84	LN80-6797	35
Pella	80	Preston	30
LN80-7532	75	Chamberlain	30
CN 210	75	L80-4323	25
Century	75	Lawrence	25
Hobbit	70	BSR 201	25
Pella 86	70	Hack	25
HC80-1946	70	Hardin	25
M74-462	70	LN80-16017	25
Harper BC	65	Gnome 85	25
A8	65	Elgin	20
HW8039	60	HC80-1944	20
BSR 101	60	Sprite	20
A80-144024	60	C1627	20
LN80-10508	60	A80-244003	15
HC80-1756	55	Sherman	15
HW8223	55	A78-227016	15
HC81-2104	55	Fayette	10
A83-271027	50	Pioneer 9271	10
Sparks	50	Williams 82	10
CN 290	50	Altona	10
HC74-634RE	50	Hoyt	10
Elgin BC	50	Pioneer 3981	5
NKS 1492	45	Cumberland	5
A83-271010	45	HA82-168018	5 5
A79-236003	45	PI 437.833	5
Lakota	45	PI 92.718-2	5
HW8371	45	L25A	5
L27	40	A82-267015	5
Harper	40		
Asgrow 3127	40		
Average	42		
LSD (0.05)	43		

 $^{^{+}}_{
m Rating}$ = 0 no reddening present; 100 reddening to tip of leaf.

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2) Inheritance of resistance to *Phytophthora megasperma* f. sp. *glycinea* in the soybean PI 92.718-2.

Phytophthora root rot, caused by Phytophthora megasperma f. sp. glycinea Kuan and Erwin, (Pmg) is one of the most serious diseases of soybeans when conditions are favorable (Kuan and Irwin, 1980). Resistance in soybeans to phytophthora root rot has been reported to be controlled by nine dominant genes (Layton et al., 1984). The cultivars 'Bombay' and 'OAC 211' also have been identified to carry one and two genes for Pmg resistance, respectively, although these genes have not been named (Moots et al., 1983). Twenty-four races of the pathogen have been identified (Keeling, 1984).

The plant introduction (PI 92.718-2) was found to have desirable agronomic traits when it was included in a plant introduction evaluation. Subsequent Pmg evaluation (unpublished data) revealed that PI 92.718-2 was resistant to the identified races 1-16 of Pmg. The objectives of this study were to determine the inheritance of resistance in PI 92.718-2 and how the resistance relates to other identified Rps genes.

<u>Materials and methods</u>: Remnant F_2 seed and progeny from individual F_2 plants from crosses with PI 92.718-2 were used in this study. The susceptible soybean lines 'S1492' A79-236003, and LN80-9452; and 'Hack' LN78-2678, and A78-227015 with the Rps_1 gene, were crossed with PI 92.718-2. Soybean lines L77-1585 with the Rps_2 gene; K1044 with the Rps_3 gene; PI 86.050 with the Rps_1^C Rps_4 genes; and 'Altona' with the Rps_6 gene were crossed with PI 92.718-2 during the summer of 1983. The F_1 seeds were planted in the greenhouse the following winter, and the F_2 seeds were planted in the field in 1984. Progeny from these F_2 plants were used in this inheritance study.

Approximately 110 F₂ seedlings from each cross to be tested were inoculated. The progeny of between 70 and 100 F₂ plants, approximately 14 F₃ seedlings, were inoculated to confirm segregation ratios obtained in the F₂ population, or to determine the relationship between the resistance in PI 92.718-2 and known $_{PD}$ genes. Seedlings were grown in sand in the greenhouse for 10 days, then inoculated with Pmg zoospores, produced as described by Moots et al. (1983), at a concentration of 10^5 per ml using the hypodermic inoculation technique (Schwenk et al., 1979). Five days after inoculation, seedlings were classified as resistant (no infection symptoms) or susceptible (hypocotyl collapse and death). The data were analyzed using the chi-square test for goodness of fit to expected ratios.

Results and discussion: The F_2 populations from the crosses of the susceptible lines with PI 92.718-2 segregated in a 15 resistant:1 susceptible ratio for races 1 and 8, indicating that two dominant genes were present in PI 92.718-2 for resistance to each race of Pmg (Table 1). The segregation of F_2 populations, when inoculated with Pmg races 2, 3, 4, and 6, indicated two or three genes in PI 92.718-2 for resistance, since it was not possible to differentiate between the ratios of 15:1 and 63:1, resistant to susceptible. The F_2 populations segregated in a ratio of 3 resistant:1 susceptible to races 5, 7, 9, and 16, which indicates that PI 92.718-2 has a single dominant gene for resistance to these races.

The F_2 populations were all resistant in the crosses involving lines carrying the Rps_1 gene with PI 92.718-2 when inoculated with Pmg races 1 and 2. This indicates that one of the genes controlling resistance to races 1 and 2 in PI 92.718-2 is allelic to Rps_1 . The gene in PI 92.718-2 may be Rps_1 or another Rps_1 allele resistant to races 1 and 2. When inoculated with race 4, the F2 population segregated in a ratio of 15:1. This indicates that PI 92.718-2 has two dominant genes for resistance to race 4, since Rps_1 conditions a susceptible reaction to race 4. This supports the race 4 data in the cross of PI 92.718-2 with S1492.

Segregation of progenies from F_2 plants are presented in Table 2. In the cross of PI 92.718-2 with the susceptible soybean lines, inoculated with Pmg race 1, the ratio indicated that three dominant genes are controlling resistance in PI 92.718-2. This differs from the F_2 results which indicated two genes for resistance to race 1. The F_2 population gave good fit to a 15 to one ratio, although five fewer plants in the susceptible class would have given good fit to a 63 to one ratio. The difference observed between the F_2 and F_3 results probably was due to sampling error in the F_2 population. When inoculated with races 3, 4, and 5, the segregation of the F_2 progeny indicated that three genes in PI 92.718-2 control resistance to race 3, two genes control resistance to race 4, and a single gene for race 5. This supports the F_2 data for races 4 and 5, and indicates that the 63 to 1 ratio with race 3 was correct.

In the cross of L77-1585 (Rps_2) with PI 92.718-2, the segregation of the F_2 progeny indicated that there were four dominant genes controlling resistance; the Rps_2 gene in L77-1585, and three genes in PI 92.718-2. This also indicates that none of the three genes for resistance in PI 92.718-2 is allelic to Rps_2 .

The segregation of F₂ progeny in the cross of K1044 (Rps_3) indicated three genes controlling resistance to race 4: the Rps_3 gene in K1044, and two genes in PI 92.718-2. The two genes controlling resistance to race 4 in PI 92.718-2 are also not allelic to Rps_3 .

The PI 86.050 has the genes ${\it Rps_1}^{\it C}$ and ${\it Rps_4}$, but only ${\it Rps_4}$ conditions the resistant reaction to race 4. In the cross of PI 86.050 with PI 92.718-2, the ${\it F_2}$ progeny, when inoculated with race 4, were all resistant. This indicates that one of the two genes in PI 92.718-2 controlling resistance to race 4 is allelic with ${\it Rps_4}$. It cannot be determined if the gene in PI 92.718-2 is identical or allelic to the gene in PI 86.050.

The progeny of F_2 plants from the cross of Altona with PI 92.718-2 were all resistant when inoculated with race 4. This indicates that the other gene

Table 1. Segregation of F2 populations from crosses of PI 92.718-2 with soybean lines carrying the rps and Rps_1 genes inoculated with races of $Phytophthora\ megasperma\ f.\ sp.\ glycinea$

		Number of	plants†		χ²
Cross	Race	R	S	Ratio [‡]	probability
rps x	1 §	104	7	15:1	0.990-0.975
PI 92.718-2	2 §	108	2	15:1 63:1	0.100-0.050 0.900-0.750
	3 [§]	118	4	15:1 63:1	0.250-0.100 0.250-0.100
	4 [§]	105	13	15:1	0.050-0.025
	5 [¶]	94	25	3:1	0.500-0.250
	6 [#]	126	3	15:1 63:1	0.100-0.050 0.500-0.250
	7 [¶]	68	34	3:1	0.100-0.050
	8 [#]	104	16	15:1	<0.050
	9#	101	23	3:1	0.100-0.050
	16	98	25	3:1	0.250-0.100
Rps ₁ x	1 ++	78	0	R	
PI 92.718-2	2 ^{‡‡}	124	0	R	
	4 ^{‡‡}	107	6	15:1	0.750-0.500

 $^{^{\}dagger}$ R = resistant and S = susceptible.

 $[\]dagger_{\rm Ratio}$ = resistant to susceptible and R = resistant.

Susceptible parent was S1392.

 $^{^{\}P}$ Susceptible parent was A79-236003.

 $^{^{\#}}$ Susceptible parent was LN80-9452.

 $^{^{++}}_{\mbox{\it Rps}_{1}}$ parent was A78-337015.

 $^{^{\}dagger\dagger}_{Rps_{1}}$ parent was Hack.

Table 2. Segregation of progenies from F₂ plants from crosses of PI 92.718-2 with soybean lines carrying the rps, Rps_2 , Rps_3 , Rps_1^C Rps_4 , and Rps_6 genes inoculated with races of Phytophthora megasperma f. sp. glucinea

	Number	of :	F ₂ р	lants	+		χ ²
Cross	Race	R		Seg.	S	Ratio [‡]	probability
rps x PI 92.718-2	1 §	66	89	23	3	37:26:1 63:1	0.100-0.50 0.250-0.100
	3 [§]	74	92	18	0	37:26:1 63:1	<0.005 0.250-0.100
	4 [¶]	38	86	48	10 10	7:8:1 15:1	0.250-0.100 0.100-0.050
	5 [¶]	12	51	39	19 19	1:2:1 3:1	0.500-0.250 0.750-0.500
L77-1585 (<i>Rps</i> ₂) x PI 92.718-2	1	78	99	21	1	175:80:1 256:1	0.100-0.050 0.750-0.500
K1044 (<i>Rps</i> ₃) x PI 92.718-2	44	74	100	26	0	37:26:1 63:1	<0.005 0.500-0.250
PI 86.050 (Rps ₁ ^C Rps ₄) x PI 92.718-2	4	97		0	0	R	
Altona (<i>Rps</i> ₆) x PI 92.718-2	4	82		0	0	R	

 $^{^{\}dagger}$ R = resistant, Seg. = segregating, and S = susceptible.

 $^{^{\}dagger}$ Ratio = homozygous resistant to segregating to homozygous susceptible or combined resistant and segregating categories to susceptible.

Susceptible parent was S1492.

[¶]Susceptible parent was A79-136012.

in PI 92.718-2 resistant to race 4 is allelic to Rps_6 . It again cannot be determined if the gene controlling resistance to race 4 is identical to the gene Rps_6 in Altona or is allelic to Rps_6 .

The data suggest that PI 92.718-2 has three genes for resistance to Pmg races 1 and 3; two genes for resistance to races 4 and 8; and a single gene controlling resistance to races 5, 7, 9, and 16. Results from inoculating plants in the F2 populations suggest that three genes in PI 92.718-2 control resistance to race 2; and 2 genes to race 6, although these results were not verified by inoculating progeny from F2 plants. Data also indicate that resistance genes in PI 92.718-2 are allelic to Rps1, Rps4, and Rps6. The gene allelic to Rps1 in PI 92.718-2 is probably not Rps1b or Rps1k. The gene Rps1b conditions the susceptible reaction to race 2. The F2 population of the cross Hack (Rps1) with PI 92.718-2, when inoculated with race 2, were all resistant, indicating that the Rps1b is not carried by PI 92.718-2. The data also indicate that PI 92.718-2 carries two genes for resistance to race 4 that are allelic to Rps4 and Rps6. Since Rps1k conditions resistance to race 4, it is not possible for PI 92.718-2 to carry Rps1k, because this would be a third gene for resistance to race 4.

Any number of hypothetical gene combinations can be proposed to fit the pattern of PI 92.718-2. Although the data are not sufficient to completely describe the inheritance of resistance, PI 92.718-2 can still be used in breeding programs to help diversify the resistance to Pmg in adapted cultivars. It also is hoped that this information may aid in further studies into the inheritance of resistance in PI 92.718-2.

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1) Tests for linkage of seed protein genes Lx_1 , Lx_3 , and Cgy_1 to markers on known linkage groups.

A project to assign linkage groups to seed protein genes on the basis of phenotypic markers was begun in the summer of 1981. F₂-segregation data for several combinations of phenotypic marker genes and storage protein loci were generated. As priorities have forced the scope of the project to be scaled back considerably, the segregation data obtained to date are reported.

Materials and methods: Crosses were made between lines containing mutant alleles for the seed protein gene loci Lx_1 , Lx_3 , and Cgy_1 and lines that had alternate alleles for one or more of the following phenotypic marker genes: T/t (linkage group 1), R/r (2), Ln/ln (4), Dt_1/dt_1 (5), L_1/l_1 (5), L_2/l_2 , i^2/i (7), W_1/W_1 (8), Lf_1/lf_1 and Lf_2/lf_2 . F_1 plants were selfed to obtain F_2 seeds. A small portion of the cotyledon was removed with a razor blade for analysis; and the remaining portion of the embryo was planted. The cotyledon chips were analyzed electrophoretically (Kitamura et al., 1983, 1984) or immunologically (Kitamura et al., 1983) to determine presence or absence of the mutant proteins. The F_2 plants were scored for presence or absence of phenotypic markers. A chi-square test for goodness of fit to a 9:3:3:1 ratio was performed.

<u>Results</u>: Evidence for linkage was not obtained. However, the possibility of loose linkages in many of the gene combinations tested where the progeny number was low could not be excluded.

Table 1. F2 dihybrid segregation ratios for seed protein gene loci Lx_1 , Lx_3 , or Cgy_1 and several phenotypic marker genes

		Phenotypic F2 segregation ratios 1,2				
Gene loci	Parental genotypes	SP	Sp	sP	sp	χ^2
Cgy ₁ ,Pc ³	cgy ₁ cgy ₁ pcpc x Cgy ₁ Cgy ₁ PcPc Keburi x T41	17	7*	12*	1	5.2
	$\mathit{Cgy}_1\mathit{Cgy}_1\mathit{PcPc} \times \mathit{cgy}_1\mathit{cgy}_1\mathit{pcpc}$ Calland x Keburi	31	10*	8*	1	2.0
Cgy ₁ ,Dt	<i>Cgy1Cgy₁DtDt</i> x <i>cgy₁cgy₁dtdt</i> Calland x Keburi	24	10*	5*	4	2.3
$^{Cgy}_1,^L_2$	Cgy_1Cgy_1L_2L_2 x cgy_1cgy_1l_2l_2 Calland x Keburi	10	9*	4*	1	5.6
Cgy_1, T	$Cgy_1Cgy_1TT \times cgy_1cgy_1$ tt Calland x Keburi	8	5*	1*	3	6.1

Table 1. Continued

		Phenotyp gregatio		1,2		
Gene loci	Parental genotypes	SP	Sp	sP	sp	χ ²
Cgy_1 , I	Cgy ₁ Cgy ₁ ii x cgy ₁ cgy ₁ i ⁱ i ⁱ Calland x Keburi	16	10	7	1*	3.2
Cgy_1 , Ln	$Cgy_1Cgy_1lnln \mathbf{x} cgy_1cgy_1LnLn$ T41 x Keburi	17	7	11	2*	3.2
Cgy ₁ ,₩	Cgy ₁ Cgy ₁ ww x cgy ₁ cgy ₁ WW T41 x Keburi	16	3	4	1*	1.2
Lx_1,I	Lx ₁ Lx ₁ i ⁱ i ⁱ x 1x ₁ 1x ₁ ii Ichigowase x PI 408.251	13	3*	5*	0	1.9
Lx_1,R	$Lx_1Lx_1rr \times lx_1lx_1RR$					
	Ichigowase x PI 408.251 T255 x PI 408.251	$\frac{8}{3}$	8 1 9	3 _0 _3	2* 0 2*	5.3
$^{Lx}_{1}$, $^{Lf}_{2}$	Lx ₁ Lx ₁ lf ₂ lf ₂ x lx ₁ lx ₁ Lf ₂ Lf ₂ T255 x PI 408.251	3	2	1	0	1.1
L_{3} , I	lx3lx3i ⁱ i ⁱ x Lx3Lx3ii					
	Ichigowase x PI 408.251 Ichigowase x Calland	15 53 68	$\begin{array}{c} 3 \\ \underline{20} \\ \overline{23} \end{array}$	$\frac{3}{7}$	0 <u>9</u> *	7.3
Lx ₃ ,Dt	$1x_31x_3$ dtdt x $1x_31x_3$ DtDt					
	Wasenatsu x L69-4659 Ichigowase x Calland Wasenatsu x L68-152	27 57 <u>23</u> 107	15* 13* <u>7</u> * 25*	18* 9* 10* 37*	5 5 3 13	0.2
$^{Lx}3^{,L}1$		23	8	7	5*	2.2
$^{Lx}3^{L}_{2}$	1x3 ¹ x3 ^L 2 ^L 2					
	Wasenatsu x L69-4659 Ichigowase x Calland Ichigowase x T245 Wasenatsu x T255	32 61 51 15 159	10 12 16 6 44	18 11 17 <u>12</u> 58	4* 5* 7* <u>3</u> *	2.1

Table 1. Continued

		Phenotypic F ₂ segregation ratios 1,2				
Gene loci	Parental genotypes	Sp	Sp	sP	sp	χ2
Lx_3, W	1x31x3ww x Lx3Lx3WW					
	Wasenatsu x L69-4659	29	10*	11*	8	
	Ichigowase x Calland	27	8*	9*	1	
	Wasenatsu x T245	35	14*	12*	4	
	Wasenatsu x T255	33	10*		6	
		124	42*	52*	19	3.3
Lx_3, Lf_1		15	7	5	5*	5.3
Lx_3 , Lf_2		35	15	12	5*	6.0

Phenotypic segregation ratios are reported in the following order: SP (doubly dominant), Sp (singly dominant, seed protein gene), sP (singly dominant, marker gene), and sp (doubly recessive).

Table 2. Estimated recombination frequencies for glycinin and $\beta\text{--conglycinin}$ subunit gene loci and lipoxygenase isozyme gene loci

Isozymes or Subunits ¹	Gene loci ²	N	±R	Source data
α, α	Cgy ₁ ,Cgy ₂	268	50 ± 3	Davies et al., 1985
α,β2	Cgy ₁ ,Cgy ₃	82	50 ± 6	Davies et al., 1985
α,β2	Cgy ₂ ,Cgy ₃	82	1 ± 1	Davies et al., 1985
A ₅ A ₄ B ₃ ,α'	${\it Gy}_4, {\it Cgy}_1$	12 0 249	45 ± 6 53 ± 4	Kitamura et al., 1984 Davies and Nielsen (unpublished)
$A_5A_4B_3$, β_2	$_{Gy_4}$, $_{Cgy_3}$	205	54 ± 4	Davies and Nielsen (unpublished)
	Gy_4,Gy_5	20	50 ± 11	Cho and Nielsen ³ (unpublished)

 $^{^2\}mathrm{Numbers}$ followed by * represent individuals with recombinant phenotype resulting only from independent segregation or crossing over between the two gene loci.

 $^{^3}$ The hairs of Keburi exhibited a flattened and curled shape which resembled the phenotype controlled by the recessive gene pc (Bernard and Singh, 1969). This phenotype segregated in a 3:1 ratio among the F_2 progeny from crosses with Calland and T41. Tests for allelism to known pc genotypes have, however, not been made.

Table 2. Continued

Isozymes or Subunits	Gene loci ²	N	±R	Source data
A _{1b} B ₂ ,A _{1b} B _{1b}	Gy1,Gy3	21	47 ± 11	Cho and Nielsen ³ (unpublished)
	Gy1,Gy4	20	43 ± 11	Cho and Nielsen ³ (unpublished)
$A_{1b}B_2, A_3B_4$	Gy1,Gy5	21	50 ± 11	Cho and Nielsen ³ (unpublished)
	Gy3,Gy4	20	36 ± 10	Cho and Nielsen ³ (unpublished)
A _{1b} B _{1b} , A ₃ B ₄	Gy3,Gy5	21	50 ± 11	Cho and Nielsen ³ (unpublished)
L ₁ ,L ₂	$^{Lx}1,^{Lx}2$	74	< 1	Davies and Nielsen (unpublished)
L ₁ ,L ₃	$^{\dot{L}x}1,^{Lx}3$	247	53 ± 5	Davies and Nielsen (unpublished)
L ₂ ,L ₃	$_{Lx_{2},Lx_{3}}$	75	43 ± 9	Davies and Nielsen, 1986
L_1,α^{\dagger}	$_{Lx_{1},Cgy_{2}}$	228	48 ± 4	Davies and Nielsen (unpublished)
L ₃ ,α'	Lx_3 , Cgy_2	228	46 ± 4	Davies and Nielsen (unpublished)

¹Identification of glycinin subunits is based on protein sequence studies with subunits from cultivar CX635-1-1-1 (Staswick et al., 1981). Variability in primary structure exists among cultivars.

²Fisher and Goldberg (1982) used the symbols G_1, G_2 , and G_3 which correspond to Gy_1 , Gy_2 , and Gy_3 , respectively. Gyl and Gy2 are tandemly linked (Fisher and Goldberg, personal communication).

 $^{^3}$ Based on restriction fragment length polymorphisms between the cultivars Forrest and Raiden. DNA probes containing coding sequence for ${\it Gy}_1$ or ${\it Gy}_5$ (cDNA probes) or ${\it Gy}_3$ (genomic DNA subclone) were hybridized to DNA from ${\it F}_2$ plants from Forrest'x Raiden crosses. Leaf DNA was purified by the method of Dellaporta et al. (1983), except that CsCl2 density gradient centrifugation was used as an additional purification step. Three to 5 $\mu {\it g}$ of digested DNA was transferred to nylon membranes by a modification of the procedure described by Scallon et al. (1985). Prehybridization and hybridization were performed under conditions where cross-hybridization of the related probe DNAs were observed to be negligible (5x SSC; 50% formamide at 55°C; SSC in 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0). The membrane was washed at 60°C in 0.1 x ssc, 0.1% SDS. Additional experimental detail will be contained in Cho, T-J. Ph.D. thesis. Purdue University, West Lafayette, IN 47907 (in preparation).

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Rapid screening for soybean lines potentially capable of biparental inheritance of plastid DNA.

<u>Introduction</u>: Shortly after the rediscovery of Mendel's work in 1900, Correns (1909) reported the maternal inheritance of a chlorophyll deficiency in *Mirabilis jalapa* and Baur (1909) described biparental transmission in *Pelargonium zonale*. Since then, the pattern of plastid transmission has been determined genetically in some 60 species of angiosperms (see Kirk and Tilney-Bassett, 1978; Tilney-Bassett and Abdel-Wahab, 1979, and references therein). Recently, there have been genetic reports that indicate that plastids are inherited maternally in *Glycine max* (Palmer and Mascia, 1980; Shoemaker et al., 1985).

The use of fluorescence microscopy to study developmental processes in pollen is evolving into a powerful tool (Coleman and Goff, 1985; Hough et al., 1985). Recently, we reported a DNA-specific fluorochrome/epifluorescence microscopy protocol that permits rapid screening for plant species potentially capable of biparental inheritance of plastid DNA (Coleman et al., 1986). The purpose of the study reported herein is to present the results of our screening of soybean germplasm sources, as well as commercial cultivars, for lines potentially capable of biparental transmission of plastid DNA using the fluorochrome/epifluorescence methodology.

Methods and materials: Soybean plants were grown in the greenhouse facilities at Brown University. Mature pollen grains were collected from flowers, fixed in ethanol:acetic acid (3:1), and subjected to analysis as previously described by Coleman and Goff (1985). Pollen grain preparations were stained with 0.05 $\mu\text{g/ml}$ 4',6-diamidino-2-phenylindole (DAPI) in McIlvaine's buffer (pH 4) and DAPI-DNA fluorescence was revealed by using a Zeiss photomicroscope equipped with a 100 W mercury lamp to deliver excitation light and the Zeiss 48-77-02 combination of excitation and emission filters. DNase-treated controls served to monitor the specificity of the staining for DNA. Plant species potentially capable of paternal transmission of plastid DNA were scored positive if cytoplasmic DNA aggregates were observed in the generative cell of mature pollen grain preparations. At least 50 pollen grains from two plants were examined from each soybean line.

Results: Preliminary results obtained from seven cultivars or accessions of G. max, and one accession of G. soja, indicate that plastids are inherited maternally (Table 1). In none of the pollen grain preparations examined was there any cytoplasmic DNA in the generative cells.

<u>Discussion</u>: Our lab has been developing the use of DNA-specific fluorochromes for studying nuclear and chromosome morphology in developing pollen (Coleman and Goff, 1985). DAPI, the fluorochrome most used in our investigations, recently has been used to detect putative plastid DNA in generative and/or sperm cells of pollen (Coleman et al., 1986). We have observed tiny DNA aggregates in the cytoplasm of the generative or sperm cells in mature pollen grains from plant species previously determined genetically to have biparental inheritance of plastids. No such DNA aggregates were observed in

Table 1. Soybean lines from which mature pollen grains were subjected to DAPI/epifluorescence microscopy

Latin name	Cultivar or accession	Cytological evidence for mode of plastid inheritance
G. max	N53-3494	maternal
o. max	PI 157.469	maternal
	Arlington	maternal
	Asgrow 5474	maternal
	Essex	maternal
	Kent	maternal
	Peking	maternal
G. soja	407.205	maternal

virtually all of the plant species studied in our lab which were known via prior genetic studies to have maternal inheritance of plastids. The cytological results from our lab have corroborated 27 out of 30 genetic studies done previously to determine plastid inheritance patterns.

We decided to use the fluorochrome/epifluorescence microscopy protocol on soybean for the following reasons: (i) soybeans are an economically important crop; (ii) there is tremendous variability between genera within the Fabaceae for mode of plastid transmission. Cytological evidence indicates that 4 out of 13 genera are potentially capable of biparental inheritance; and (iii) from our cytological evidence, we believe there may be variation within some species for the pattern of plastid inheritance. The basis for this third point may be that retention of organelle DNA through pollen development is a developmental trait that may be under the control of only one or a few nuclear genes.

The knowledge gained by our investigation of plastid inheritance patterns may have a significant impact on crop improvement. Breeders seeking to manipulate organelle genomes should profit immediately. The significance of our preliminary findings warrants our continued investigation of soybean germplasm potentially capable of biparental transmission of plastid DNA. Presently, we have a number of G. \max lines, as well as other species of Glycine, growing in the greenhouse, and we would be willing to examine other lines, if seed were sent.

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1) Analysis of active transposable element systems in soybean.

Since the identification of an insertion element (Tgml) in the lectin gene (Le1) of lectin-negative soybean lines (Goldberg et al., 1983; Vodkin et al., 1983), this laboratory has been interested in identifying and characterizing active transposable element systems of soybean. Tgml exhibits the structural features of known transposable element and appears to be related to the En/Spm elements of corn and Taml of snapdragon (Rhodes and Vodkin, 1985). However, there is no evidence for mobility of Tgml and the Le-phenotype is stably inherited.

To approach this problem, we have been analyzing various mutants that exhibit mottled or variegated phenotypes of the type expected from transposable-element action. These include: (1) the $Y18^m$ allele at the Y18 locus, which conditions a pattern of yellow and green sectors on the leaves; (2) the r^m allele at the R locus, which conditions a pattern of black rings and spots on an otherwise brown seedcoat; and (3) the allelic series (I,i^k,i^i,i) at the I locus, which determines the distribution of pigmentation over the seedcoat. Types of analyses include classical genetic studies, mutagenic studies as well as molecular genetic studies. A brief description of some of the more interesting results is as follows:

Genetic studies: 1. We have observed somatic and germinal instability of the r^m allele at the R locus. Somatic instability is observed when single plants derived from our r^m stock produce a mixture of seeds that are both black and mottled (r^m) . Sometimes, the mutation to black is confined to a single branch or a few branches, but on other occasions, the black and mottled seed are found scattered randomly on the plant. Both types of seed have been found within a single seed pod. No consistent pattern of mutability has been observed. The described instability is strictly somatic since when the black seed from such plants are grown they produce plants with mottled seed. Germinal instability is observed, however, when single plants derived from the r^m stock produce all black seeds. When these seeds are planted and grown, they produce plants with all black seed or they segregate 3:1 for black: r^m . We also have observed plants giving rise to all brown seed which then breed true for brown. Analysis of this instability is continuing.

- 2. Lines have been derived from F_2 populations of an Le^- X r^m cross that are both Le^- and have r^m seedcoats. Two such lines have produced F_4 populations that exhibit mutability of seedcoat color. One line produced a plant that had a seed mixture of black, brown, and r^m . The other line produced a plant that had all black seed. Subsequent generations can now be screened for reversion events of Le^- to Le^+ due to excision of the Tgml element from the lectin gene possibly induced by an interaction of an active transposable element with Tgml.
- 3. This past summer, we identified a spontaneous mutation in 'Sooty', a Le^- cultivar. The mutant plant produced a single branch that exhibited the yellow/green sectoring of leaves characteristic of the $Y18^m$ mutant.

Seed from this branch were grown in the greenhouse during the fall of 1986 and they segregated roughly 3:1 for green:yellow lethal. The defect appears to be different than that caused by $Y18^m$ since the yellow plants derived from $Y18^m$ can survive under identical conditions in the greenhouse. Only one leaflet on one plant showed any somatic instability in the form of yellow/green sectoring.

Mutagenic program: 1. Le^+ , Le^- , $Y18^m$ and r^m lines of soybean have been exposed to 20 kR and 25 kR doses of gamma radiation (^{60}Co) as a form of genomic stress to induce transposable element activity. M1 plants and M2 populations have been analyzed so far. One Le^- line studied frequently produces $Y18^m$ -like mottling on single branches of M1 plants. However, this sectoring does not seem to be inherited. An M2 population of 'Williams' (Le^+) segregated for leaf color variegation which resembles $Y18^m$. Out of approximately 100 seed planted, 2 gave rise to plants that were very stunted. One of these plants produced a branch with variegated leaves; the other plant was variegated throughout. Approximately 10 to 20 seed were collected per plant. Some of these were grown in the greenhouse during the fall of 1986 and about half of them produced plants that continued to mottle. No yellow lethal plants have yet been observed.

Molecular genetic studies: 1. Southern blot analysis of genomic DNAs using Tgml-specific probes is being conducted on various lines harboring the different alleles present at the Y18, R, and T loci. We are trying to determine whether there is an association of the instability of the alleles at these loci with genomic rearrangements of Tgml-related sequences. Preliminary results suggest that we are observing some rearrangements in the black-seed derivatives of the r^m stock as compared to the original DNA. However, more detailed analysis is in progress.

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 Genetics of reaction to soybean mosaic virus (SMV) in cultivars exhibiting differential reaction to SMV strains.

A series of seven differential strain groups of SMV, labelled G1 to G7, were reported by Cho and Goodman (1979). Buzzell and Tu (1984) added another strain, G7A, and Lim (1985) added an additional strain, C14. We have been studying the genetic relationships among the cultivars that define the strain groups. Our intial studies involved the cultivars 'Marshall', 'Kwanggyo', 'York', 'Ogden', and PI 96.983, which differentiate the seven strain groups of Cho and Goodman.

In the field in 1986, we planted F3 lines from crosses among the differential cultivars, including two resistant x susceptible crosses, and inoculated them with SMV isolate SMV-VA, which has been classified into Cho and Goodman's strain group G1 (Hunst and Tolin, 1982). The methods used were described by Roane et al. (1983). Counts of plants with and without symptoms were made for each $\rm F_3$ row and these data were used to classify each row as hymozygous resistant, segregating, or homozygous susceptible. The results from the crosses evaluated in 1986 are in Table 1.

In a previous report (Roane et al., 1986), we indicated that the progeny from the cross PI 96.983 x Essex segregated as though two dominant, independently segregating genes for resistance were present. The data we obtained this year from a similar cross, PI 96.983 x Lee 68, indicate segregation for only one dominant gene. The reason for this contradiction is not clear at this point. It is possible that there are genes in one of the susceptible parents that interact with a resistance gene in PI 96.983. However, that does not appear to have a high probability, since the cross of Essex x Marshall cited in the previous report and the cross of Marshall x Lee 68 (Table 1) both fit a 1:2:1 F2 ratio. Another possibility is that PI 96.983 is not completely uniform. We will continue to evaluate these crosses.

Kiihl and Hartwig (1979) established that the resistance genes in PI 96.983 and Ogden are alleles at the same locus. We reported (Roane et al., 1986) that the dominant genes for resistance in Kwanggyo and Marshall appeared to be allelic. In our 1986 nursery, we observed one or two symptomatic plants in a number of rows from the resistant x resistant crosses (Table 1). In previous years, we have tested similar plants with ELISA (Lommel et al., 1982) and found a negative reaction for SMV. ELISA testing was not available this year, but it seems safe to assume that the 1986 plants also were infected by viruses other than SMV. The apparent lack of segregation in all of the resistant x resistant crosses supports our previous work and is an indication that, in all of the resistant cultivars in this study, the resistance genes are alleles at a single locus. Similar results could be obtained with very close linkage among all the genes, but it seems very improbable that five separate genes (one from each resistant parent) would all be tightly linked. Three crosses (Ogden x York, Ogden x Marshall, and Ogden x Kwanggyo) have not yet been evaluated, but we predict that these crosses would give similar results. We are continuing to evaluate the reaction of the complete set of crosses among these parents for reaction to other strains of SMV.

Table 1. Reaction of F3 lines to SMV-VA

			Classes			
Cross	Cross type	Homo res.	Seg.	Homo. susc.	1:2:1 x ²	Р
PI 96.983 x Lee 68	(RxS)	33	55	28	0.741	.5070
Marshall x Lee 68	(RxS)	35	80,	26	3.709	.1020
PI 96.983 x York	(RxR)	77	25	0		
York x Marshall	(RxR)	92	14,	0		
York x Kwanggyo	(RxR)	32	3	0		
Kwanggyo x Marshall	(RxR)	56	2++	0		
PI 96.983 x Ogden	(RxR)	60	8++	0		
PI 96.983 x Marshall	(RxR)	142	3++	0		
PI 96.983 x Kwanggyo	(RxR)	41	1++	0		

Only 2 rows fit 3:1; all others had 1 or 2 symptomatic plants.

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 $^{^{++}}$ All rows had only 1 or 2 symptomatic plants.

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l) Response of tolerant and susceptible soybean cultivars to Columbia lance nematode.

Columbia lance nematode, CLN [Hoplolaimus columbus Sher], is a migratory endo- and ectoparasite of soybean and commonly causes yield reductions of up to 30% in tolerant cultivars. Complete crop failure may occur on moisture-stressed or susceptible cultivars. First recognized as a major parasite of cotton and soybean in 1967 (Fassultiotis et al., 1968), CLN now infests a large portion of the coastal plain of South Carolina, Georgia, and North Carolina.

No sources of resistance to CLN are known, although tolerance has been demonstrated in some soybean cultivars by comparing relative seed yield of nematicide-treated and nontreated plots (Nyczepir and Lewis, 1979). We are interested in screening Plant Introduction materials for new sources of tolerance or resistance to CLN in addition to studying inheritance of tolerance in known sources; however, we have not known what measurements or criteria to use to assess CLN damage in single plants or segregating populations. The objective of this study was to examine tolerant and susceptible cultivars of soybean growing in a CLN-infested field to determine plant evaluation criteria that may be associated with tolerance.

Materials and methods: Plots of CLN-tolerant 'Foster', 'Centennial', and 'Coker 368', and susceptible 'Braxton' were planted with and without inrow subsoiling in a CLN-infested field near Blackville, SC, on July 17, 1986. Five single plants (replications) were randomly excavated from a predetermined l-m-row area at 5, 7, and 9 weeks after planting. Due to extreme heat and drought conditions during 1986, growth stages were compressed and, therefore, all cultivars matured together.

Each plant was rated on a scale of relative lateral-root branching from one (least) to five (most), and darkened or necrotic root lesions were counted. CLN were extracted from roots by using a modified Seinhorst mist apparatus and expressed per gram of tissue. Shoots were dried and weighed.

Results: CLN g root⁻¹ measurements in subsoiled plots were highly variable and no significant differences existed at either 5 or 9 weeks after planting; however, Braxton was significantly higher than the tolerant cultivars at 7 weeks (Table 1). In nonsubsoiled plots, where infestation and damage is greatest, CLN g root⁻¹ did not follow predicted tolerance at 5 weeks, but Braxton had values significantly higher than all tolerant cultivars at 7 weeks and higher than Foster and Centennial at 9 weeks. This measurement is sensitive to sampling time and may not be adequate to assess tolerance under field conditions. Under controlled conditions, where age of nematodes and generation time may be more closely controlled, CLN g root⁻¹ may be a useful measurement.

CLN often feeds on secondary root primordia, which may induce lateral root branching (Lewis et al., 1976), and the branching score we used reflected cultivar tolerance well at 5 and 7 weeks in subsoiled plots. Braxton had

Table 1. Columbia lance nematodes per gram of root, branching score, and shoot and root weights for tolerant and susceptible soybean cultivars grown with and without in-row subsoiling and sampled at five, seven, and nine weeks after planting, 1986

		- Subso	iled			- Nonsul	osoiled ·	
Cultivar	CLN g root-1	Branch 1 to 5	Shoot g	Root g	CLN g root-1	Branch 1 to 5	Shoot	Root
		Five	weeks a	after pl	anting			
Foster	106.3	1.0	0.51	0.49	324.1	4.0	0.18	0.53
Centennial	222.3	1.8	0.15	0.27	557.9	4.2	0.49	1.29
Coker 368	92.1	2.4	0.41	0.55	143.4	3.6	0.57	1.37
Braxton	272.5	3.0	0.28	0.41	282.3	4.4	0.57	1.37
LSD ^a	NS	0.5	0.08	NS	93.5	0.2	0.34	0.27
CV (%)	142	39	46	54	62	11	38	43
		Sever	weeks	after pl	lanting			
Foster	94.4	2.2	0.62	4.30	92.7	3.0	0.94	5.00
Centennial	93.0	2.4	0.79	3.44	42.3	4.2	2.18	5.60
Coker 368	63.7	3.0	2.13	4.80	22.2	3.2	0.97	3.92
Braxton	190.6	4.4	0.99	4.06	130.3	4.0	1.39	4.52
LSD	37.1	0.5	0.42	NS	27.2	0.3	0.31	NS
CV (%)	61	15	62	28	59	9	37	28
		Nine	weeks a	fter pl	anting			
Foster	52.7	2.2	1.85	1.44	108.9	2.6	1.07	1.86
Centennial	57.9	3.0	1.30	1.19	81.9	3.0	3.08	2.51
Coker 368	55.0	2.2	2.52	1.24	183.6	2.6	0.41	0.94
Braxton	27.1	3.2	1.60	2.73	206.5	2.8	0.55	1.36
LSD	NS	NS	NS	0.40	41.3	NS	0.63	0.38
CV (%)	77	30	58	37	53	33	69	36

 $^{^{\}rm a}{\rm Least}$ significant difference (0.05 level) calculated only with a significant F test.

significantly higher branching values than the tolerant cultivars and Foster (most tolerant) had the lowest values. We plan to study the utility of this measurement more closely in the greenhouse and in nematicide versus nonnematicide-treated field plots. Branching scores were less reliable for predicting tolerance in nonsubsoiled plots, possibly indicating that extremely high levels of infestation and damage overcome tolerance mechanisms.

Shoot and root weights did not appear to be useful in comparing cultivar tolerance, and may reflect differences between the cultivars themselves regardless of CLN. Shoot and root weights may have more utility when studied under controlled conditions or in fumigated versus nonfumigated plots. Nyczepir and Lewis (1979) demonstrated that individual soybean genotypes usually have higher shoot and root weights when grown in fumigated soil than in CLN-infested soil. Lesions (data not shown) did not correlate to CLN g root⁻¹ measurements and were difficult to discern, possibly due to the presence of other organisms that also cause lesions.

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Screening of the USDA soybean cultivar collection for slow and fast urease isozymes.

Urease present in soybean seed is known to consist of two isozyme variants. These variants are referred to as electrophoretically fast or slow on polyacrylamide gels and are believed to be polymers of a single subunit (Buttery and Buzzell, 1971; Polacco and Havir, 1979). The slow variant, considered a hexamer, has been estimated at 480,000 daltons by Polacco and Havir (1979) and at 520,000 daltons by Buttery and Buzzell (1971). Although data on the fast form of urease is limited, it is believed to be a trimer of approximately 205,000 daltons (Buttery and Buzzell, 1971). Kloth and Hymowitz (1985) have presented evidence that the urease isozymes are codominantly inherited. They assigned the gene symbol Eul-a to the slow-moving allele and Eul-b to the fast-moving allele.

Polacco (1985, personal communication) screened about 8500 soybean plant introductions for the presence or absence of urease activity. However, he did not determine which soybean cultivars contain the fast or slow urease variants. The objectives of this study were (1) to screen the USDA soybean cultivar collection (Canada and USA public releases) for urease isozymes; and (2) to determine if there are positive associations between type of electrophoretic banding pattern and maturity group, geographical origin or year of release.

The 397 soybean cultivars used in this study were obtained from R. L. Bernard, USDA, Urbana, Illinois. Seed extracts and seed urease isozymes were determined by the procedure of Kloth and Hymowitz (1985).

The distribution of seed urease variants in soybean cultivars by their year of introduction or release up to 1985 is summarized in Table 1. Analysis of 397 cultivars revealed nearly equal numbers of those containing slow and fast seed urease variants. The slow variant was about twice as prevalent as the fast variant in soybean cultivars released through 1939. Approximately 90% of the 31 soybean cultivars whose origin was Japan contained the slow urease allele.

The soybean cultivars from China (48) and Korea (16) were about equally divided between the slow and fast urease variants. The soybean cultivars released from 1940 through 1969 contained about an equal number of fast and slow seed urease variants. However, of the 110 cultivars released since 1970, 70% contain the fast variant of seed urease. Furthermore, 84% of the 44 cultivars released since 1980 contain the fast isozyme. Thus, an apparent reversal on the predominant form of seed urease has occurred over time, whereby the fast variant has become prevalent in the newly released soybean cultivars.

An analysis of recently released cultivars that contain the slow variant reveals that 15 of 18 released since 1976 contain 'Harosoy' or a derivation of Harosoy in their pedigree. Harosoy, a cross between 'Mandarin (Ottawa)' and 'A.K. (Harrow)' was released in 1951 (Hymowitz et al., 1977),

and its seed contains the urease slow variant. Thus, the majority of recently released public soybean cultivars containing the seed urease slow variant can be traced back to a narrow germplasm pool.

Table 2 contains the distribution of seed urease variants in $297\ \mathrm{soy-}$ bean cultivars by maturity group. No significant conclusions can be drawn from the data.

Table 1. Distribution of seed urease variants in soybean cultivars by year of introduction or release

	Urease	form	
Year	Fast	Slow	Total
-1899	1	3	4
1900-1909	8	19	27
1910-1919	6	12	18
1920-1929	20	41	61
1930-1939	12	21	33
1940-1949	24	24	48
1950-1959	14	16	30
1960-1969	32	31	63
1970-1979	40	26	66
1980-	37	7	44
Total	194	203 ^a	397 ^a

 $[\]ensuremath{^{\mathrm{a}}}\xspace\mathrm{Date}$ of release information not available for three cultivars containing the slow isozyme of urease.

Table 2. Distribution of seed urease variants in soybean cultivars by maturity group $% \left(1\right) =\left(1\right) \left(1\right) +\left(1\right) \left(1\right) \left(1\right) +\left(1\right) \left(1\right) \left$

Maturity	Ure	ase		
group	Fast	Slow	Total	
000	0	1	1	
00	3	18	21	
0	11	12	23	
I	26	21	47	
11	28	37	65	
III	45	24	69	
IV	42	31	73	
V	8	9	17	
VI	18	10	28	
VII	9	14	23	
VIII	3	25	28	
IX	1	1	2	
X	0	0	0	
Total	194	203	397	

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2) Screening of the USDA Glycine soja collection for urease variants.

Two isozyme variants of seed urease are present in soybeans. These variants are referred to as electrophoretically fast and slow on polyacrylamide gels (Buttery and Buzzell, 1971; Polacco and Havir, 1979). Kloth and Hymowitz (1985) demonstrated that the urease isozymes are codominantly inherited. They assigned the gene symbol Eul-a to the slow-moving allele and Eul-b to the fast-moving allele.

Of about 8500 soybean accessions screened for the presence or absence of seed urease activity (Polacco, 1985, personal communication), four accessions were identified having a seed urease null phenotype: PI 229324 ('Itachi') (Polacco et al., 1982) and PI 416975 ('Kairyou Kakushin'), PI 417073 ('Kounou 1'), and PI 417074 ('Kounou 2') (Kloth et al., 1987). The absence of urease activity apparently is controlled by a null lesion (sun) and is linked to Eul by approximately one map unit. In the presence of Sun, Eul-a or Eul-b is expressed in soybean seed (Kloth et al., 1987).

No screening of the *Glycine soja* Sieb. and Zucc. seed for urease isozyme variants has been done. *Glycine soja* is the wild annual ancestor of the soybean. The objectives of this study were (1) to screen the USDA G. soja collection for the slow and fast urease isozymes and (2) to determine if there were any null phenotypes within the collection.

The 629~G. soja accessions used in this study were obtained from R. L. Bernard, USDA, Urbana, Illinois. Seed extracts and seed urease isozymes were determined by the procedure of Kloth and Hymowitz (1985). Seed of G. soja lacking urease bands were checked for urease activity by the urease color test described by Kloth (1985).

Results of the G. soja screening for seed urease banding pattern are summarized in Table 1. Five accessions (PI 407074, 407117, 407118, 407119, 407120) lacked seed urease. These five accessions came from Japan. It is of interest to note that the four soybean accessions lacking seed urease also came from Japan. The rest of the 624 G. soja accessions analyzed contained the slow variant.

Table 1. Distribution of seed urease variants and urease nulls in the USDA Glycine soja germplasm collection

		- Urease form-		
Origin	Fast	Slow	Null	Total
China	0	97	0	97
Japan	0	177	5	182
Korea	0	314	0	314
Taiwan	0	2	0	2
USSR	0	34	0	34
Total	0	624	5	629

The slow urease band in *G. soja* (PI 391.587) migrated to the same position as soybean cultivar 'Columbia', the slow urease band standard. In addition, an artificial hybrid (mixture of seed extracts) containing both PI 391.587 and Columbia formed a single slow urease band.

From these data, it appears as though Japan is the geographical origin of seed lacking urease. The null phenotype only was found in G. \max and G. soja seed from that country. The absence of the fast urease variant in the 629 G. soja accessions screened is intriguing. Perhaps the fast urease variant was derived as a mutational event in the domesticated soybean.

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3) Isozymes as predictors of ploidy level in Glycine tabacina (Labill.) Benth.

Glycine tabacina (Labill.) Benth. and G. tomentella Hayata have both diploid ($2\underline{n}=2\underline{x}=40$) and tetraploid ($2\underline{n}=4\underline{x}=80$) forms (Singh and Hymowitz, 1985a). Although the diploid plants can be distinguished cytologically from the tetraploids, the procedure is tedious, and requires precious greenhouse space.

Seed isozyme analysis may prove to be a simpler approach for differentiating between the diploid and tetraploid tabacinas. Grant et al. (1984) have demonstrated that zymogram patterns were predictive of ploidy level in G. tomentella. Specifically, they reported that shikimate dehydrogenase and endopeptidase were good predictors of ploidy level. However, the isozymes could not distinguish between the 38 vs. 40 or the 78 vs. 80 G. tomentella cytotypes.

The objective of this investigation was to identify the ploidy level of *G. tabacina* accessions of unknown chromosome number in order to facilitate the organization of the germplasm collection for further cytological and genetic studies. Thirteen recently introduced *G. tabacina* accessions from Australia were used in the study. Two known accessions, a diploid tabacina (PI 339.661) and a tetraploid tabacina (PI 193.232) were used as checks. Five dry seed per accession were used in the analysis. The extraction, starch gel electrophoresis and staining procedures were carried out according to the procedure outlined by Menancio and Hymowitz (n.d.). The enzymes used were: (1) alcohol dehydrogenase (ADH); (2) malate dehydrogenase (MDH); (3) phosphoglucoisomerase (PGI); and (4) shikimate dehydrogenase (SDH). The choice of enzymes was based upon screening data obtained from 68 *G. tabacina* accessions of known chromosome number.

Three out of the four enzyme systems used were able to differentiate between the (6) diploid and (7) tetraploid tabacina accessions of unknown chromosome number. These were MDH, PGI and SDH. ADH yielded single bands for diploids but certain tetraploids exhibited this same pattern. The disappearance of an MDH band in the multibanded region of the MDH pattern (see Broué et al., 1977) was found to be diagnostic of diploid accessions. Similarly, the SDH patterns described by Grant et al. (1984) for G. tomentella were likewise observed among the diploid vs. tetraploid tabacina accessions. The greater number of bands and more complicated patterns of PGI were to a certain degree predictive of the tetraploid form. The increased number of bands observed in the tetraploid tabacinas suggest that these forms probably contain more than one genome. These findings are concordant with the cytogenetic evidence of Singh and Hymowitz (1985b) that tetraploid G. tabacina is an allopolyploid complex.

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4) Examination of wild perennial Glycine species for glyphosate tolerance.

There has been much recent interest in the development of crops that are tolerant or resistant to the herbicide glyphosate. Glyphosate is a nonselective systemic herbicide that can control many annual and perennial weeds regardless of their size, a characteristic atypical of most selective herbicides used in crop production. Crop tolerance to glyphosate would be beneficial as it would provide growers, for the first time, the opportunity to control almost all weeds with a single herbicide application. The wild progenitors of cultivated crops have in the past provided favorable genetic traits that often can be incorporated into these crops. We have been screening accessions of wild perennial *Glycine* species for tolerance to glyphosate. The existence of tolerance in wild germplasm could lead to the development of a glyphosate-tolerant soybean cultivar.

Materials and methods:

Greenhouse Studies were conducted to evaluate 173 accessions of the following seven Glycine species for glyphosate tolerance: G. canescens, G. clandestina, G. cyrtoloba, G. falcata, G. latifolia, G. tabacina, and G. tomentella. Seeds were nicked with a razor blade for scarification, and germinated on moist filter paper at 25 C until radicles emerged. Germinated seeds were planted in plastic tubes with a volume of 175 cm³ containing a growth medium of two parts soil, one part vermiculite, and one part sand. These species grow slowly for an extended period following emergence, after which they begin to grow more rapidly. Glyphosate was applied to plants when they reached the rapid growth stage, since systemic herbicides are most effective when plants are most actively growing.

Glyphosate rates of 0.28 to 0.56 kg/hectare (kg/ha) are sufficient to kill soybeans, while rates of at least 0.56 kg/ha are required for weed control. In initial studies, glyphosate was applied to plants at a rate of 0.28 kg/ha and plants visually rated for injury two weeks following application. Surviving plants were retreated with 0.56 kg/ha and rated as before. Glyphosate application continued in a sequential manner in 0.28 kg/ha increments to a rate of 1.4 kg/ha. Accessions tolerant to 1.4 kg/ha were replanted and treated with a 2.2 kg/ha rate of glyphosate. Accessions surviving 2.2 kg/ha were replanted and treated with 1.1 and 2.2 kg/ha of glyphosate to confirm previous observations. Glycine max, 'Williams 82', was included in the latter study as a comparison.

Cell culture. Experimental materials included G. max 'Asgrow 3127', G. tabacina and G. cyrtoloba. Glycine tabacina and G. cyrtoloba seeds were surface-sterilized by passage through 30% commercial bleach (5.25% sodium hypochlorite) for 30 minutes and rinsed three times in sterile distilled water. Seeds were germinated aseptically on agar-solidified nutrient medium. Approximately 10-day-old seedlings were used to provide 1-cm hypocotyl pieces for inoculation onto modified PC-L2 agar medium described by Phillips and Collins for callus production. Adjustments to the PC-L2 were addition of 2,4-D, NAA and kinetin at 0.4, 4.7 and 2.2 mg/liter, respectively, and elimination of picloram. Two- to four-month-old callus was used to initiate cell suspension cultures by inoculating 1.0 to 1.5 g tissue into ca. 10 ml of callus initiation media except that the 2,4-D concentration was increased to 1.2mg/l. Cell suspensions were subcultured weekly by using 5 ml of suspension to inoculate 40 ml of fresh media in 125 ml Erlenmeyer flasks. After one week, cell densities averaged 0.3 g fresh wt/ml. Cultures were rotated at 130 rpm under continuous indirect fluorescent light at 27 C. A two-year-old cell suspension of G. max was obtained from Dr. Jack Widholm (University of Illinois) and maintained under conditions described above in PC-L2 with 2,4-D at 0.4 mg/l and no picloram. Glyphosate treatments were prepared by adding 2.5 ml glyphosate stock to 40 ml of fresh media prior to subculture. Glyphosate stocks contained 4.8 g MES/1 and pH adjusted to 5.8. Cell fresh weights were obtained one week after treatment.

Results and discussion:

In greenhouse studies, differential response of accessions to glyphosate was observed among and within species. All species contained at least one accession able to survive a glyphosate rate of 1.4 kg/ha (Table 1). Differences among species became more apparent, however, upon consideration of the relative injury sustained by accessions in response to this glyphosate rate. Accessions of G. clandestina, G. cyrtoloba, and G. tabacina were the only plants to survive with less than 20% injury. Some accessions of G. falcata and G. tomentella survived with between 20% and 40% injury, along with additional accessions of the previous three species. Accessions of G. canescens and G. latifolia were least tolerant to glyphosate, since none survived 1.4 kg/ha glyphosate with less than 40% injury.

Treatment of the most tolerant accessions with 2.2 kg/ha of glyphosate resulted in the survival of plants from two G. cyrtoloba accessions and three G. tabacina accessions. When these five accessions were replanted and rescreened, all survived 1.1 kg/ha of glyphosate with 50% injury or less (Table 2). However, all plants except those in one G. tabacina accession were killed at the 2.2 kg/ha rate. While results in the last two studies were not entirely consistent, we feel tolerance was demonstrated, especially since all G. max plants were killed at 1.1 kg/ha of glyphosate.

Table 1. Response of Glycine spp. accessions to 1.4 kg/ha of glyphosate

Species	Number screened	Number surviving	<40% injury	<20% injury	
G. tabacina	69	60	15	3	
G. clandestina	35	20	7	2	
G. tomentella	29	24	4	0	
G. canescens	22	4	0	0	
G. latifolia	11	3	0	0	
G. cyrtoloba	5	3	1	1	
G. falcata	2	1	1	0	

Table 2. Response of five tolerant Glycine spp. accessions and G. max to 1.1 and 2.2 kg/ha of glyphosate

		% Inj	ury ———
Species	Accessions	1.1 kg/ha	2.0 kg/ha
G. tabacina	A	50	63
G. tabacina	В	45	100
G. tabacina	C	43	100
G. cyrtoloba	A	20	100
G. cyrtoloba	В	30	100
G. max	'Williams 82'	100	100

Table 3. Response of Glycine spp. cell suspension cultures to glyphosate

Glyphosate	Fresh weight of cells (% of control)			
(μmolar)	G. cyrtoloba	G. tabacina	G. max	
100	95	18	75	
250	47	13	31	
500	41	0	23	
1000	25	0	0	

Cell suspension cultures were initiated for one accession each of *G. tabacina* and *G. cyrtoloba*. These two accessions showed the greatest tolerance to glyphosate in greenhouse studies. Cell fresh weights of *G. cyrtoloba* were greater than those of *G. max* at all glyphosate concentrations (Table 3). However, differences in the fresh weights between *G. cyrtoloba* and *G. max* cells at the four concentrations ranged from only 16 to 25%. Differences of this magnitude may not be sufficient to attribute tolerance observed at the whole plant level entirely to biochemical factors, and indicate that differential uptake and translocation of herbicide from the leaf surface may be partially responsible for tolerance.

Glycine tabacina cells were less tolerant of glyphosate than those of G. max. This difference in response was inconsistent with greenhouse studies, where the order of tolerance was reversed. These data would tend to support a hypothesis that, for some accessions, tolerance differences observed at the whole plant level are due to uptake and translocation differences, rather than differences at the active site of the herbicide, the enzyme EPSP-synthase.

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5) Preliminary investigations on the salt tolerance of wild perennial Glycine species.

In 1983, 1984, and 1985, the senior author collected wild perennial <code>Glycine</code> species in the South Pacific (New Caledonia, Vanuatu, Tonga) and in Australia (Queensland including Great Barrier Reef islands, Western Australia, Northern Territory). The collecting trips were sponsored by the Rockefeller Foundation and the International Board of Plant Genetic Resources. The latter collecting trips were conducted jointly with scientists from CSIRO/Canberra. Seed of <code>Glycine tabacina</code> and <code>G. tomentella</code> were collected from plants growing on sandy or coral beaches. It seemed reasonable to assume that some of these accessions were tolerant of salt.

Two preliminary growth-chamber experiments were conducted to assess possible salt tolerance of some wild perennial Glycine accessions. Seeds were sown in sand culture, with nutrient solution (Hoagland #2 with micronutrients) pumped through at 3-hour intervals (Hoagland and Arnon, 1950). The plants were grown at 24 C, 10 hours light at 300 $\mu \rm Em^{-2} s^{-1}$.

In the first experiment, synthetic seawater, equivalent to natural seawater in concentrations of chloride, sodium, magnesium and sulfur, was added to the nutrient reservoirs at the time of first seedling emergence and in three additional steps at six-day intervals, to give final concentrations equal to either 10 or 30% of that of natural seawater (Weast, 1981-1982).

The final harvest was 135 days after planting. All plants produced seeds (Table 1).

In the second experiment, either 17 or 34% synthetic seawater was imposed at once after the seedlings emerged. The cultivar 'Williams' replaced $G.\ cyrtoloba$. The only plants to survive the 34% synthetic seawater were $G.\ latifolia$ and $G.\ tabacina$.

Table 1. Effect of two concentrations of synthetic seawater on the growth of six *Glycine* species

	II.	Seawater co	ncentration
Species			30%
		gra	ams ^a
G. canescens	401	7.3	11.6
G. clandestina	425	4.2	3.5
G. cyrtoloba	545	4.1	3.1
G. latifolia	359	33.1	7.7
G. tabacina	643	4.2	1.7
G. tomentella	553	15.9	18.0

^aTotal plant dry weight, 6 plants.

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1) Genotype-race interactions in relation to phytophthora rot of soybean.

Many of the widely grown soybean cultivars in the Mid-Atlantic region are susceptible to phytophthora rot caused by *Phytophthora megasperma* f. sp. *glycinea* Kuan and Erwin (Pmg). There is limited information available on tolerance to Pmg in these popular soybean cultivars. Tolerance or field resistance to Pmg in soybean is characterized as race nonspecific resistance involving root resistance to Pmg infection, whereas resistance to Pmg in soybeans is characterized as race specific resistance (Schmitthenner, 1985). The objective of this study was to evaluate race-specific resistance and levels of tolerance to phytophthora rot in soybean cultivars grown in Maryland.

Materials and methods: Greenhouse screening tests were performed on public and private soybean cultivars entered in the state variety trials. The greenhouse evaluations used 14-day-old lima bean agar cultures of Pmg grown at 25 C. Race-specific resistance to Pmg was determined by placing mycelia in a hypocotyl slit approximately 1 cm below the cotyledonary node of 10-day-old seedlings. Inoculated plants (5 plants per 10-cm pot) were kept in a moist chamber for 24 hrs. Root resistance (tolerance) was determined by transplanting 4-day-old seedlings into 10-cm plastic pots (5 plants per pot) filled with steam-pasteurized compost media infested with a mycelial Pmg suspension. Soil was infested 24 hours before transplanting with one petri plate culture per pot. Culture medium was placed on wet soil surface and chopped into pieces approximately 0.5 cm square, then worked into the top 5 cm of soil. Cultivars were compared and rated for number of live plants 2 to 3 weeks after transplanting. The transplant technique has produced tolerance ratings among cultivars which are comparable to those obtained using a greenhouse mycelium inoculum layer technique similar to that described by Walker and Schmitthenner (1984). The transplant procedure ensured selection of uniform seedlings and reduced potential complications caused by abnormal, diseased seedlings. To account for both plant kill and plant stunting caused by phytophthora rot, cultivars grown with and without Pmg were compared for number of live plants, and for average plant height (i.e., growth above the cotyledons) at 21 days after planting (equation - Table 3). A virulent isolate of race 1 of Pmg from Maryland was used to determine hypocotyl and root resistance. Cultivars exhibiting root resistance to race l from Maryland were subsequently evaluated for root resistance against isolates of races 3, 4, 5, 7, 10, 12, 13, 14, 15, 16, and 24 and additional isolates of race 1 obtained from Dr. B. J. Keeling, USDA-ARS, Stoneville, MS, and J. D. Paxton, Univ. of Illinois, Urbana.

Results: Many of the early (group III) and mid-maturity (group IV) cultivars entered in the variety trials showed race-specific resistance to isolates of Pmg race l (race l is currently the only race reported in Maryland). However, there was little race-specific hypocotyl resistance to Pmg found in late maturity (group V) soybean cultivars with only two of the 15 public and private lines tested showing resistance to race l isolates from Maryland (Table l). Eight of the 13 group V cultivars without race-specific

Table 1. Hypocotyl and root resistance reactions to race l (Maryland isolate) in Maturity Group V cultivars entered in the state soybean variety trials $^{\hat{a}}$

Group V	Cultivars	Hypocotyl resistance	Root resistance
Public	Essex	S	S
TUDITE	Bay	S	S
	York	S	R
	Forrest	S	R
	Epps	S	R
	Toano	S	R
Private	Asgrow 5149	S	MR
	Coker 425	S	MS
	Coker 575	S	MR
	Coker 485	S	MR
	Jacques X-83140	S	MR
	Pioneer 5482	S	S
	Pioneer 9531	R	NA
	S. S. FFR-561	S	MS
	Stanford HT 5203	R	NA

^aS = all plants dead (5 plants per pot);

Table 2. Root resistance reaction to different races of ${\rm Pmg}^{\rm a}$

			Cultivars		
Races	Verde	Essex	York	Ware	Forrest
1 (MD)	S	S	R	R	R
3	-	S	R	R	R
4	S	S	R	R	R
7	MS	MR	R	R	R
10	S	S	S	MR	MR
14	-	S	R	R	R
16	MS	MR	R	R	R
24	S	S	S	R	MR

aS = all plants dead (5 plants per pot);

MS = 3-4 dead;

MR = 3-4 alive;

R = no dead;

 $^{{\}tt NA}$ = unable to evaluate root resistance because of hypocotyl resistance for race 1.

MS = 3-4 plants dead;

MR = 3-4 plants alive;

R = no dead.

Table 3. Root resistance ratings of four soybean cultivars to different races of $\ensuremath{{\rm Pmg}}^a$

	Cultivars				
Isolate/Race ^b	Essex	Williams	York	Ware	
Test 1		%			
MD-1	3 ^c	0	98	100	
IL-1	75	76	95	97	
IL-5	14	26	87	92	
K-10	0	2	9	65	
K-24	7	32	23	85	
LSD (0.05)	15	25	27	22	
Test 2					
MD-1	4	1	92	96	
IL-1	81	83	96	102	
IL-5	43	56	109	97	
K-10	0	1	3	87	
K-24	22	29	54	99	
LSD (0.05)	20	22	34	30	

^aRating =
$$\frac{\text{(no. of live plants)} [\bar{x} \text{ height (Pmg)}]}{\text{(no. of live plants)} [\bar{x} \text{ height (w/o Pmg)}]} \times 100\%$$

 b MD-1 = isolate of race l from Maryland; IL-1 and IL-5 = isolates of race l and 5, respectively, from Illinois; K-10 and K-24 = isolates of race l0 and 24, respectively, from Mississippi.

 $^{\text{C}}\text{Values}$ shown are the means of four replications; 5 plants per replication.

resistance to race 1 exhibited high levels of root resistance to race 1 isolates of Pmg. However, 'Essex', the most widely planted late-maturing cultivar in Maryland, showed little or no root resistance (Table 1).

The results in Tables 2 and 3 indicate that there are different levels of root resistance in soybean to Pmg. The genotype race interactions were highly significant. The isolates of race 1 from Illinois and Maryland showed marked differences in virulence (Table 3). The race 1 isolate from Illinois may have lost virulence; however, cultures of race 1 from Maryland have shown no loss in virulence over an 18-month period. The isolates of races 10 and 24 from Mississippi also appeared to differ in virulence. The reactions of 'York', Essex and 'Williams' to Maryland race 1 isolates compared to their

reactions to either the race 10 or 24 isolates would indicate a difference in pathogenicity rather than virulence. 'Ware' and 'Forrest' showed tolerance to all isolates.

In future breeding efforts, soybean cultivars should be selected for the highest levels of Pmg tolerance available. Preliminary findings indicate that selecting for tolerance to a limited number of Pmg isolates may not necessarily ensure the highest levels of root resistance.

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Linkage of the Rps2 and Rj2 genes for resistance to Phytophthora and Rhizobium.

The 'Harosoy' Rps2 isoline L70-6494 appears to have the Rj2 allele for ineffective nodulation with strains in the l22 and c1 serogroups of Rhizobium japonicum. Devine and Breithaupt (1981) screened some germplasm and uniform group test lines for the presence of Rj2 and Rj4. The line L75-l1806 (in 1979 preliminary IV) was reported to have Rj2. It probably also had Rps2 since it was rated resistant in the group test results, and came from L70-6494 x 'Williams'. The source of Rj2 and Rps2 was CNS (Caldwell, 1966; Kilen et al., 1974).

Advanced backcross lines with Rps2 and their source lines were obtained from R. L. Bernard, Univ. of Illinois, Urbana, and T. C. Kilen, Stoneville, MS. Their results with Phytophthora were considered to be reliable, so no inoculations were done. I inoculated the lines followed by (122) in Figure 1 with the USDA strain 122 to determine the presence of Rj2. Identical results were obtained with USDA 7 (in the cl serogroup) in Beltsville by R. C. Leffel. The change of N48-1394 to D51-4863 coincides with the move of E. E. Hartwig from North Carolina (N) to Mississippi (D). Figure 1 shows that Rj2-Rps2 remained associated through quite a few segregating and backcross generations. The probability of this occurring with independent genes would be rather small. The 'Clark' backcross line L77-2060 nodulated normally with both 7 and 122, which probably represents a crossover.

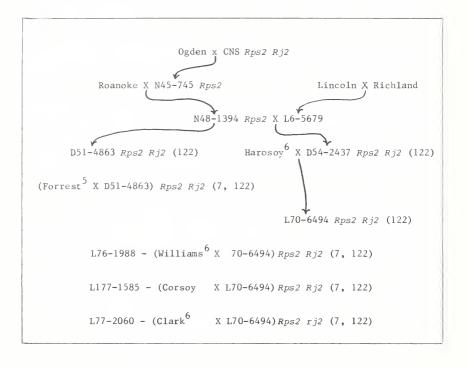


Figure 1. Pedigrees of lines to show transmissions of Rps2-Rj2 from CNS. The (7, 122)s indicate the USDA Rhizobium strains used to show Rj2 v. rj2

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1) The use of gradient PAGE gels to study soybean isozymes.

Soybean isozymes have been studied genetically primarily with simple PAGE and starch gel electrophoresis (see Rennie et al., 1986, for a comparison and Kiang and Gorman, 1983, or Palmer et al., 1985, for reviews). Our objective was to test if the use of vertical gels with a PAGE concentration gradient would enhance isozyme resolution over simple horizontal PAGE gels. Gradient gels were tested for their ability to resolve: amylase (Am), acid phosphatase (AP), Diaphorase (Dia), glucose-6-phosphate dehydrogenase (GPD), 6-phosphogluconate dehydrogenase (PCD), phosphoglucomutase (PCM) and superoxide dismutase/tetrazolium oxidase (SOD/TO) isozymes.

We have found that the use of 3% to 12% gradients significantly improved the resolution of certain soybean isozymes over that obtained with simple 7%, horizontal PAGE gels. The vertical arrangement allows for a larger sample to be loaded into the gel, improving the resolution of weakly staining isozymes, while the concentration gradient causes proteins to "stack" as they migrate, resulting in sharper bands. The gradient also enhances molecular sieving, allowing for greater separation of conformational or size isozymes. However, vertical gradient gels in general are not as economical or as practical for germplasm screening as are thick horizontal PAGE or starch gels.

Materials and methods: We used precast gradient gels obtained from Integrated Separation Systems (44 Mechanic St., Newton, MA 02164). These gels were 16x12x1.5 cm in size, with a linear gradient running from 3% at the top of the gel to 12% at the bottom cast in Margolis buffer (0.09 M Tris, 0.08 M boric acid, 0.0026 M EDTA and 0.005% sodium azide pH 8.3). We found that using a 3% stacking gel in 0.125 M Tris-HCl (pH 6.8) buffer not only facilitated sample loading, but it also enhanced protein stacking. The samples used were the clear supernatant (at least two 20-min centrifugations at 12,000 g, with filtration through glass wool until a clear supernatant is obtained) of overnight-soaked soybean seeds, less their seed coats. The seeds were ground to a thick slurry in 1 ml/g of cold Margolis or stacking gel buffer with a mortar and pestle. Each sample was then diluted to 10 mls/g for filtration and centrifugation. After centrifugation, 5% (wt/vol) sucrose and 0.25% bromophenol blue was added to each sample from a 10x stock solution made in Margolis buffer. Only about a third of one seed is needed to have a sufficient sample, so that the remainder can be germinated. A sample, $100 \mu l$, was loaded with a syringe into each sample well (10/gel), which are formed by placing a comb in the stacking gel before it polymerizes. The gels were electrophoresed in a 4°C cold room at 150 volts (constant voltage) for 4-8 hr (depending on the isozyme) using Hoefer "studier" gel apparatus and Margolis tank buffer. Staining was done as described by Gorman (1983).

Results: The most significant improvement in resolution was obtained for Am and SOD/TO isozymes, where several additional isozymes not observed in horizontal PAGE gels were resolved (see subsequent newsletter articles). In addition, the zymogram bands were much sharper. A fast-migrating AP band (an additional band faster migrating than the variable Ap-a, Ap-b or Ap-c allele products), not usually resolved in horizontal gels was clearly visualized in the gradient gels. The products of the Pgml gene, which stain weakly

on horizontal gels, were much more distinct on gradient gels, but their migration was faster than the Pgm2 gene's product. This is opposite from what was observed in simple gels, suggesting that the Pgml protein has a smaller configuration and weaker charge than the Pgm2 protein. This would result in the Pgml protein having a faster mobility in the gradient gel where molecular sieving plays a more important role, but slower migration in the simple gel where charge is more important. PGD zymogram patterns were similar between the gradient and simple gels for the faster migrating bands, but the slower variable bands (products of the Pgd gene) were not well-resolved in the gradient gels. This may be because of the high background staining observed or because the variable bands are caused by charge differences which the simple gel does a better job in separating. GPD zymograms were similar between gradient and simple gels (neither does a good job of resolving distinct bands). No diaphorase bands were detected on gradient gels for unknown reasons. The PGM, PGD and Dia gradient zymograms need further testing along with other enzymes we have not yet tested.

In conclusion, it appears that gradient gel systems may be useful for answering certain specific questions regarding soybean isozymes and for providing additional molecular information on the difference between isozyme variants. However, the greater cost, lower number of samples/gel, increased amount of sample preparation, and the ability to stain only for one enzyme/sample makes gradient gels impractical for most labs to use for large-scale germplasm screening.

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2) Soybean amylase zymograms on gradient PAGE gels.

There have been numerous papers dealing with amylase isozymes in soybeans (i.e., Morita and Yagi, 1979; Hildebrand and Hymowitz, 1980; Kiang, 1981; Adams et al., 1981; Mikami et al., 1982). All have reported betaamylase as the predominant amylase type in most soybean lines and that varietal differences in beta-amylase either for electrophoretic mobility, pI, or enzyme activity were observed. Gorman and Kiang (1978), Hildebrand and Hymowitz (1980), Kiang (1981), and Griffin and Palmer (1986) reported similar results concerning the inheritance of different beta-amylase electrophoretic variants (i.e., variants were the result of five different alleles at the Spl locus). However, there has been a great deal of confusion over the number of amylase (both alpha and beta) isozymes found in soybeans and in correlating genetic electrophoretic variants with other biochemical results. A recent paper by Norby and Rinne (1985) reported 10 different varietal amylase zymograms with some varieties having as many as 18 different amylase bands. They claimed that a more sensitive staining procedure (including starch in the gel with running gels at low temperatures) allowed for detection of weak isozymes. However, the zymograms they reported have no resemblance to other reported zymograms and known genetic variants. The lines 'Chestnut' and 'Altona', which are known to have at most a trace of betaamylase (Adams et al., 1981; Hildebrand and Hymowitz, 1980), were reported by Norby and Rinne as having numerous beta-amylase isozymes with no apparent visible intensity differences as compared with other lines tested.

We felt that there was a need for further investigation to attempt to rectify the apparent confusion and inconsistencies in the soybean amylase literature. Our objective was to test gradient PAGE gels with crude samples from lines with known genetic differences in amylase isozymes to see if additional isozymes were detected. Gradient gels allow for a larger sample volume since they are run in vertical apparatus and constantly stack proteins into sharp bands. We hoped that any weak isozymes not detectable by simple electrophoresis and conventional staining might be observed in gradient gels.

Nakamura and Futsuhara (1985) have already shed considerable light with regard to beta-amylase, as they have found that the Spl-a variant corresponds to the 7 band high pI type of Mikami et al. (1982) and Spl-b corresponds to the 7 band low pI type. Further, they found, through renatured SDS gel analysis, that only a single molecular weight species of beta-amylase exists in homozygous seed. The Spl-a protein was found to have a slow SDS migration (likely due to having a higher molecular weight) than the Spl-b protein did. Unfortunatley, they did not test Altona, Chestnut or PI 132.201, the lines homozygous for the Spl-an and Spl-1 alleles. Two dimensional (isoelectric and SDS) electrophoresis of Spl-a and Spl-b variants revealed two corresponding families of isozymes with two major and two to three minor isozymes. The patterns were identical (except for placement), suggesting that isoelectric focusing is simply fragmenting the same protein into different bands for unknown reasons, or that two multigene families exist with the Spl gene acting as a regulator.

<u>Methods</u>: Samples were prepared and electrophoresed as described in the previous report, except that it was necessary to dilute samples with normal beta-amylase activity (all lines except Altona, Chestnut and PI 132.201) to 20 ml/g. Gels were stained by first soaking the gel for 1 hr

in 1% hydrolized starch in 0.2 M acetate buffer (pH 5.0). Then, the gels were rinsed with water and placed in 0.1% iodine plus 0.5% potassium iodine solution, which visualized amylase as achromatic bands in a blue background. To identify alpha-amylase from beta-amylase, varieties with known beta variants were compared and a 1% solution of soluble starch (limit dextran, which acts as a substrate only for alpha-amylase) was substituted for the hydrolized starch when staining gels. Sweet potato beta-amylase from Sigma was used as a control. We also tried to use a beta-amylase inhibitor obtained from Sigma, but this seemed to have no effect on any of the isozymes under the conditions tested.

Results: The gradient gels revealed a total of 10-11 amylase bands. Four strong beta-amylase bands were found. We believe these bands to be beta-amylase because of their intensity, the fact that they did not stain with limit dextran, and because all four were altered in comparing lines with known beta-amylase variants. The mobility of all four bands changed when comparing samples homozygous for the Sp1-a and Sp1-b alleles. The lines Chestnut and PI 132.201 (homozygous for the Sp1-an allele) displayed all four bands, but at a greatly reduced intensity, while F2 seeds from the cross PI 132.201 by Altona (sp-1 allele) segregated with some seeds having zymograms similar to the PI 132.201 and others not having any of the four bands. Thus, it appears that the four bands are all products of the same gene (Sp1). They may represent different conformational forms or result from the binding of the beta-amylase protein to unknown charged factors. Morita et al. (1976) suggested, after amino acid analysis, that conformational changes were responsible for the difference between two soybean betaamylase forms separated by ion exchange chromatography. The idea that multiple beta-amylase electrophoretic forms are conformational forms of the same protein is further supported by Nakamura and Futsuhara's (1985) finding that soybean beta-amylase is a simple protein and that there is only a single molecular weight form of beta-amylase in homozygous seeds when tested in SDS gels. Nakamura and Futsuhara (1985) observed 4-5 beta amylase bands in two dimensional gels and Morita and Yagi (1918) found four different bands with isoelectric focusing.

The two strongest of the other 10-11 bands visualized on gradient gels were clearly alpha-amylase as they stained with limit dextran. The remaining weaker bands are also likely to be alpha-amylase, but they did not stain using limit dextran as a substrate. We feel that this is probably because of their lower activity and the lower degree of resolution obtained with limit dextran. No mobility differences were observed in any of the six varieties tested for any bands other than the four beta-amylase bands. The slowest migrating band (one of the two certain alpha-amylase bands) did appear to have lower activity in some samples (Chestnut, PI 132.201 and Altona). The other putative alpha-amylase bands appeared to have the same intensity in all samples. None of the alpha-amylase varietal differences reported by Norby and Rinne (1985) were observed. However, it is clear that they are correct in suggesting that there may be additional alpha-amylase isozymes not detected on simple PAGE gels.

In conclusion, we suggest that, despite the ability to resolve multiple beta-amylase bands with isoelectric focusing and gradient gels, a single structural beta-amylase gene with five known alleles is active in soybeans. There is a possibility that some of these alleles may actually represent variation in a cis regulatory gene. It appears that soybean beta-amylase allozymes differ in electrophoretic mobility, pI and molecular weight and

may each have several conformational forms. Studies starting from crude soybean meal rather than pure seed lines (i.e., Morita et al., 1975) probably isolated more than one allozyme. Alpha-amylase isozyme patterns will require additional work before any genetic interpretation is possible.

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M. B. Gorman M. Yasenchack 3) Superoxide dismutase (SOD) and tetrazolium oxidase (TO) zymograms observed in gradient PAGE gels and preliminary inheritance data for type 3 zymograms.

We tested gradient PAGE gels for their ability to resolve isozymes with a specific SOD (EC 1.15.1.1) stain as well as with a nonspecific TO stain. In particular, we wanted to test gradient gels for use in scoring $\rm F_2$ seeds segregating for the cultivar-specific type 1 and type 3 TO zymogram patterns first reported by Gorman and Kiang (1977). TO electrophoretic bands are caused by any number of enzymes (particularly SOD) that prevent the spontaneous reduction of tetrazolium dyes in the electron-transfer staining systems used to detect dehydrogenases.

The type 3 TO pattern observed in simple horizontal PAGE gels had slower migrating bands 9 and 10 (out of a total of 11 bands) than did type 1 or 2 cultivars (Gorman and Kiang, 1977). Gorman and Kiang (1978) suggested a codominant mode of inheritance based on the observation of a few apparent heterozygous seeds with both slow and fast bands; however, genetic analysis was not done. Scoring of type 3 seeds proved to be difficult with this electrophoretic system. Gorman and Kiang (1978) reported that the type 1 zymogram was dominant to the type 2 (lacking bands 5 and 6). The type 1 and 2 TO zymograms were found to correspond with the int-oxidase zymogram variants reported by Larson and Beeson (1970). Griffin and Palmer (1984) reported observing the same three cultivar-specific types upon SOD staining of vertical PAGE gels (Davis system). However, their zymogram patterns were somewhat different, with type 2 lacking bands 4 and 5 and type 3 having slower migrating bands 8 and 9 (out of a total of 9 bands).

Methods: The electrophoretic methods described in the previous newsletter article were followed, except that samples were frozen until ready for use and then recentrifuged. The 0.125 M Tris-HCl (pH 6.8) stacking gel buffer was used to prepare samples. Only about one-third of each F_2 seed was used per sample with the rest of the seed germinated for producing an F_3 . Gels were run at 150 volts for 6-7 hr (until 1.5 hr after the bromophenol blue marker dye had run out of the gel). The gels were stained for TO as described by Gorman and Kiang (1977) and for SOD by the method of Beauchamp and Fridovich (1971).

Results: A total of 14 bands (not counting the Ep locus peroxidase band which appears on some samples) was observed for homozygous seed on TO-stained gels and 11 bands on SOD-stained gels. Four weak fast-migrating bands were observed on the TO-stained gradient gels that were not observed on simple PAGE gels, but otherwise the type 1 zymograms were the same. The type 2 gradient zymogram was found to lack the fourth and fifth bands using either stain. The type 3 zymogram in TO-stained gradient gels had a slower migrating tenth band than type 1 or 2 zymograms had. The mobility of only one band was affected, rather than two as observed in the simple horizontal or Davis systems. Contrary to the finding of Griffin and Palmer (1984), we did not observe this variable band at all on SOD-stained gels. The SOD gels also lacked the two fastest migrating bands. Band 13 was a weak band and may simply have not been resolved as well with the SOD stain, but the variable band 10 and band 14 were strong bands.

A total of 45 F_2 seeds from the cross 'Agate' by 'Kingston' were scored with TO-stained gradient gels. Sixteen were found to have the fast band

only, twenty had both the fast and the slow bands and nine had only the slow band. Fitting these observed results with a 1:2:1 codominant ratio yielded a chi-square of 2.73, which has a P value of 0.26. While the gradient gels proved to be a superior system for detecting type 3 zymograms and scoring segregating seeds than simple horizontal PAGE gels, resolution still was not sharp enough to conclude whether or not heterozygous zymograms included any intermediate (heterodimer) bands.

We are currently growing F_2 plants so that an F_3 analysis can be made, as well as making a reciprocal cross. Once this information is obtained, a gene symbol can be assigned. However, unless we are in error about type 3 variants not involving SOD isozymes or unless another specific enzyme activity is identified, the nonspecific TO designation will need to be used despite its falling into disuse in isozyme literature.

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1) Varietal differences of soybeans sensitivity to low temperature during germination.

Developing of cold-tolerant soybean varieties is one of the new main trends in soybean breeding. Such studies were started in the USA (Veitenheimer et al., 1984; Unander and Orf, 1984; Harrison and Nickell, 1984; Hillsman et al., 1977; Hicks, 1978; Seddigh and Jolliff, 1984; Littlejohns and Tanner, 1976; Hume and Jackson, 1981; Voldeng et al., 1984; Sanbuichi, 1980; Goto and Yamamoto, 1972; Schmid and Keller, 1980; Szyrmer and Janicka, 1985; Gromova, 1975; Lunin, 1981; Malysh and Bobrikov, 1984; Sherepitko and Balashow, 1985).

The experiments demonstrated that low temperatures reduced germination, field emergence, the rate of early growth and soybean yield. The same results have been obtained in our previous experiments (Sichkar and Beversdorf, 1980, 1982). Our objective in this study was evaluation of soybean varieties from world germplasm collection for cold tolerance and determination of the nature of inheritance of the character.

Materials and methods: In 1980-1985, over 500 soybean varieties from the world germplasm collection were tested for reaction to low temperatures in growth chambers. Seeds were placed on filter paper discs in petri dishes or in sand in growth boxes. The experiment was conducted at 7°, in a check it was 23 or 25°. Under low temperature regime, the number germinated seeds was counted every day. The final germination was determined on the 28th or 30th day after soaking. In the checks, germination was determined on the 7th day after the beginning of the experiments. The length of the radicles in the experiments was measured on the 28th or 30th day, in the check on the 7th day. Earlier planting in the field was carried out in the period from the end of March to the beginning of April. The optimum sowing was made at 18-20° soil temperature in seed bed.

Results and discussion: Germination of seeds from a large number of world germplasm collection of soybean made it possible to differentiate the studied forms as cold-resistant and sensitive to low temperature (Tables 1 and 2). Such varieties as 'Grant', 'Severnaya 2', 'Lincoln', 'Norchief', 'Kishinevskaya 5', 'Kievskaya 71', 'SRF 100', 'Man-Szan-tsin' and 'Hawkeye' showed high germination at 7°. Varieties such as: 'Amurskaya buraya', 'VIR-4956', 'Vengerskaya 45', 'Gieso', 'Altona' and others were sensitive to the same temperature. In another experiment such varieties as 'Comet', 'Amurskaya 41', 'Dobrudschanka 18', 'Vengerka', 'Salut 216' and 'Nairn' showed cold resistance during several seasons (Table 3). The varieties 'Chabarovskaya 33', 'Yantarnaya', Gieso and others were inferior as far as that character is concerned. Figure 1 shows germination dynamics of the seeds differing in sensitivity to low temperatures. The seeds of cold-resistant variety 'Negrutsa' showed high germination on 12th day after soaking, while first germinated seeds of Gieso variety appeared only in 24 days at low temperature.

Table 1. Germination and growth of the radicles of cold-tolerant soybean varieties $% \left(1\right) =\left\{ 1\right\} =\left\{ 1\right\}$

		Ten	perature	
	25	0	7	•
Variety	Germination energy (%)	Germination (%)	Germination (%)	Length of radicles (cm)
Grant	95	96	84	0.89 ± 0.07
Amurskaya 147	95	96	80	1.37 ± 0.09
Severnaya 2	98	99	88	1.16 ± 0.06
Lincoln	99	99	84	1.27 ± 0.06
Norchief	86	90	83	1.30 ± 0.07
Kishinevskaya 5	92	93	95	1.18 ± 0.18
Kievskaya 71	76	87	76	1.10 ± 0.06
SRF 100	96	96	92	1.22 ± 0.08
Amurskaya 400	96	92	74	0.99 ± 0.06
Man-Szan-tsin	87	89	75	0.89 ± 0.05
Hawkeye	65	86	74	0.86 ± 0.06
Steele	94	98	82	0.90 ± 0.05
Bombay	93	98	80	0.77 ± 0.04

Table 2. Germination and growth of radicles of sensitive soybean varieties

		Tem	perature ———	
	25	0		7°
Variety	Germination energy (%)	Germination (%)	Germination (%)	Length of radicles (cm)
Amurskaya 411	86	87	40	0.64 ± 0.05
Ruest	88	90	37	0.83 ± 0.10
Amurskaya buraya	96	97	18	0.77 ± 0.04
Karona	95	97	44	0.73 ± 0.06
Gieso	92	100	42	0.80 ± 0.07
Altona	69	89	37	0.67 ± 0.04
VIR-4956	97	98	38	0.88 ± 0.07
075-2 (Canada)	90	95	42	1.14 ± 0.09
Vengerskaya 48	77	91	34	0.85 ± 0.09

Table 3. Germination of soybean seeds from world collection at optimum and low temperature $% \left(1\right) =\left\{ 1\right\} =\left\{ 1\right\}$

			- Temper	ature —		
		23°			7°	
Variety	1981	1982	1983	1981	1982	1983
Amurskaya 41	91.3	97.3	93.0	96.0	97.0	87.9
Comet	92.5	98.5	91.0	90.2	96.4	93.5
Dobrudschanka 18	97.0	95.0	94.0	96.8	96.2	93.7
Negrutsa	98.0	-	96.0	95.4	94.6	91.2
Vengerka	94.6	94.5	93.5	95.2	95.1	87.6
Salut 216	92.0	90.0	93.0	92.2	91.1	80.8
Da-Li-Huan	99.0	95.5	95.5	93.9	88.7	87.1
Vytka 3	99.0	93.0	98.0	94.8	83.6	94.4
Beltskaya 25	99.0	98.0	96.0	92.8	71.8	53.0
Gieso	94.0	98.0	98.0	40.3	58.1	65.5
Dalnevostochnaya 913	99.0	98.5	96.5	91.0	56.8	70.8
Boby buryje maslichnyje	97.0	97.3	94.0	32.6	52.0	62.7
Yantarnaya	97.0	89.0	93.5	59.5	50.1	49.5
Habarovskaya 33	98.0	93.5	96.0	89.7	21.3	77.9
P 73-2	94.0	93.0	95.5	29.6	17.5	47.0

Table 4. Field emergence of soybean varieties from world germplasm collection at early sowing

	0	ptimum	sowing	3		Early s	sowing	
Variety	1981	1982	1983	Ave.	1981	1982	1983	Ave.
Amurskaya 41	-	82.0	81.0	_	-	56.6	77.0	_
Comet	-	77.0	80.5	-	-	68.7	81.8	-
Dobrudschanka 18	86.5	88.0	83.5	86.0	84.6	77.8	84.9	82.4
Negrutsa	80.0	79.5	85.0	81.5	81.0	71.3	93.7	82.0
Vengerka	84.0	86.5	84.5	85.0	77.0	69.3	85.2	77.2
Salut 216	68.0	78.0	70.0	72.0	57.9	42.1	59.1	53.0
Da-Li-Huan	75.0	88.5	86.0	83.2	67.0	55.7	91.9	71.5
Vytka 3	82.5	80.0	86.5	83.0	85.5	43.7	85.5	71.6
Beltskaya 25	82.9	84.5	81.5	83.0	53.5	45.2	53.2	50.6
Gieso	53.5	86.5	83.5	74.4	44.5	29.1	60.0	44.5
Dalnevostochnaya 913	78.5	83.5	86.0	82.7	65.8	14.9	60.7	47.1
Boby buryje maslichnyje	76.0	86.0	87.5	83.3	81.9	71.6	89.4	81.0
Yantarnaya	62.5	76.0	81.5	73.3	28.8	14.3	61.1	34.7
Altona	81.5	84.0	85.5	83.7	66.9	35.0	58.3	53.4
P 73-2	59.5	81.0	86.0	75.5	44.6	20.4	50.0	38.3

It should be noted that, in general, at 7° , cold-resistant genotypes exceeded the others in length of the radicles (Figure 2). In the first experiment, the exception was Canadian line 075-2. It showed low field germination at low temperature, and the growth of radicles was equal to that of cold-resistant genotypes.

In the second experiment, variety Comet showed the decreased radicle growth, though it was cold-tolerant during seed germination. The results suggested that some soybean genotypes showed specific reactions to low temperature.

In field conditions, such varieties as Amurskaya 41, Comet, Dobrudschanka 18, Negrutsa, and Vengerka confirmed high germination under early sowing. Low field germination of several varieties at optimum sowing date in 1981 is attributed to unfavorable temperature regime during May.

Thus, the results of laboratory and field experiments demonstrated substantial genotypic variability of soybean varieties to germinate at low temperature. It is important that the local Moldavian varieties Dobrudschanka 18 and Negrutsa showed high cold resistance. Germination of the majority of studied varieties varied in different years. Apparently, it depended on conditions in the period of seed formation.

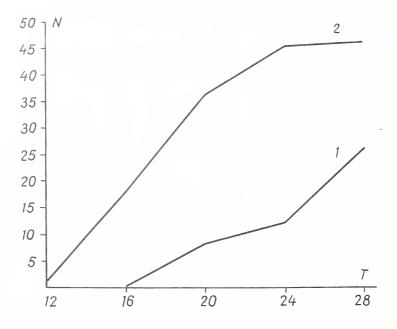


Figure 1. The germination dynamics of sensitive Gieso variety and cold-resistant Negrutsa variety under low temperature

Axis of ordinates - the number of germinated seeds (N)

Axis of abscissas - the number of days (T)

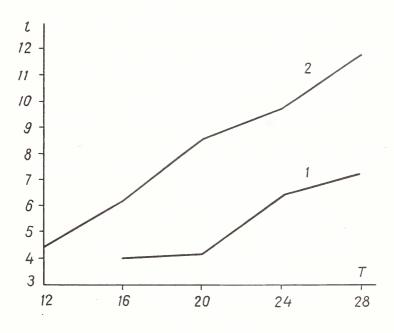


Figure 2. The growth dynamics of the radicles of sensitive Gieso variety (1) and cold-resistant Negrutsa variety (2) under low temperature

Axis of ordinates - average length of the radicles, 1, mm

Axis of abscissas - the number of days (T)

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1) Inheritance of hardseededness in soybean.

Soybean is often characterized by hardseededness or seed impermeability, when the seeds do not imbibe water and they germinate very slowly. Germination period of such seeds is very prolonged for a month or more. Hardseededness is caused by reduced water permeability of seed coat. We meet this character in many plant species, but more often in Leguminosae, Rosaceae, Lilium (Chorniy, 1980).

The number of impermeable seeds depends upon the maturation period and storage conditions. Dry air favors the hard seeds part increasing (Niko-laeva, 1982). At the same time, this character is controlled genetically; that is confirmed by essential differences between soybean species and varieties in controlled conditions (Woodworth, 1933; Kilen and Hartwig, 1978; Shahi and Pandey, 1982).

Hard seeds keep the germination for a long time. Seed coat impermeability of cultivated soybean varieties improves harvest efficiency due to nonbreakage during threshing. It may also improve seed quality by minimizing detrimental effects of adverse environmental conditions both in field and during storage. On the other hand, soybean hardseededness should be taken into account in estimating number of sown seeds to provide the necessary plant density. Hardseededness should also be taken into account in plant breeding. Plants have selected against this characteristic, because soybean hardseededness is considered an undesirable trait. There are quite limited and contradictory data in the special literature on the problem of inheritance of soybean hardseededness.

While analyzing F_2 segregation between the cultivars 'Hardee' (soft seeds) and PI 326578 (hard seeds), Srinives and Hadley (1980) revealed that parental differences were dependent on three main genes with complete domination of seed coat permeability in each locus.

Shahi and Pandey (1982) studied inheritance of seed coat permeability in two interspecific crosses ('UPSM-534' x G. formosana and Hardee x G. formosana). They came to the conclusion that the character was controlled by a single gene with impermeability domination. In intervarietal crosses, the authors revealed that permeability is dominant over impermeability.

Thus, different authors received various data on genetical determination of hardseededness, both in genes number and in allelic interaction nature. In plant breeding practice, attention is given to crossing of wild G. ussuriensis soybean lines with large number of hard seeds. So, the aim of the present work was to study the inheritance of seed impermeability in soybean hybrids.

Varieties 'Chaika' (USSR), 'Beachwood' and 'Merit' (Canada), 'Evans' (USA) and wild *G. ussuriensis* soybean were used as parental forms.

Percentage of impermeable seeds of parental forms and \mathbf{F}_1 and \mathbf{F}_2 hybrids was calculated.

Forty to 60 seeds of each plant were soaked in petri dishes on paper towel for 18 days, and then the number of unimbibed seeds was determined.

The formula of Serebrovsky in Rokitsky modification was applied to determine the number of genes controlling hardseededness (Rokitsky, 1974).

$$n = \frac{3D^2 - 4DD_1 + 4D^2}{16 \left[\sigma_{F2}^2 - \frac{\left(\sigma^2 P_1 + \sigma^2 P_2\right)^2}{2}\right]}$$

where

D = difference between average values of the parental forms;

 \mathbf{D}_{1} = difference between \mathbf{F}_{1} average characteristic and recessive type characteristic;

 σ^2 = variances of parental cultivars and F_2 .

The distribution of plant frequencies according to percentage of impermeable seeds is presented in Table ${\bf l}$.

Plants of the parental varieties Beachwood, Evans, and Chaika did not have hard seeds. The variety Merit had two plants with 25% and 5% of hard seeds, while the seeds of the other 20 studied plants were normal. Unlike cultivated varieties, hard seeds of wild ussuriensis soybean averaged 80% with variation of individual plants from 53% to 95%.

Hard seeds percentage of F_1 hybrid crosses Beachwood x wild ussuriensis soybean and Evans x wild ussuriensis soybean were 30% and 21%, respectively. Hence, the percentage of impermeable seeds of F_1 plants was intermediate between the parental varieties, somewhat similar to cultivated type.

Among 279 plants tested in the F_2 hybrids of cultivated soybean x wild ussuriensis soybean, 74 were similar to cultivated parental type, 74 were classified as wild type, and 131 plants were intermediate and each individual plant had from 1 to 50% impermeable seeds.

Using the formula presented above, it was ascertained that the number of genes controlling hardseededness was 1.34 for Beachwood x ussuriensis cross and 1.05 for Evans x wild ussuriensis cross.

Both calculated values for cross combinations and the whole experimental data made it possible for us to suppose that the character of seed permeability in studied varieties is determined by one single pair of alleles (one gene) with partial dominance of seed permeability over hardseededness. The results gave a good fit to expected ratios for a single recessive gene controlling hardseededness in soybean. The estimated degree of dominance of permeable seed coat over hardseededness was unequal in different crosses.

Hardseededness of soybean hybrids and their parental varieties Table 1.

1				%	hard	seeds	seeds/plant	t t				Marin	Average	
Cross combination and parental varieties	0	01-1	02-11	08-12	07-18	05-14	09-15	07-19	08-17	06-18	001-16	studied plants	hard seeds	0.5
Beachwood	10											10	0.0	0.0
ussuriensis							П	_	2	3	_	∞	80.6 ±4.4	12.4
F_1 Beachwood x ussuriensis													30.0	
F ₂ Beachwood x	13	10	7	9	9	4	11	2	7	3	-	70	34.2 ±3.4	28.7
Merit	20	2										22	0.34±0.24	1.14
ussuriensis							1	1	2	4		∞	74.9 ±4.6	13.0
F ₂ Merit x <u>ussuriensis</u>	18	_∞	11	9	9	5	7	4	ا ا ع	-	2	71	27.9 ±3.2	26.9
Evans	20											20	0.0	0.0
ussuriensis									-	2	П	∞	84.8 ±3.8	10.8
${f F}_1$ Evans x ussuriensis													21.0	
F ₂ Evans x ussuriensis	18	17	9	7	4	-	ا ا ع	-	ا ا ع	4	-	89	21.6 ±3.4	28.0
Chaika	10											10	0.0	0.0
ussuriensis								_	2	4	1	∞	81.2 ±3.0	8.6
${\rm F_2}$ Chaika x ussuriensis	22	9	∞	9	3	4	4	7	4	7	2	70	31.6 ±3.9	32.8
Σ F ₂	7.1	41	35	25	19	14	25	11	17	15	9	279		

Table 2. The results of plant segregation analysis of seed-coat permeability

Cross	χ²	Probability
Beachwood x ussuriensis	3.62	0.25 > p > 0.10
Merit x ussuriensis	0.83	0.75 > p > 0.50
Evans x ussuriensis	2.00	0.50 > p > 0.25
Chaika x ussuriensis	3.68	0.25 > p > 0.10

Thus, in crosses with varieties Beachwood and Evans as maternal parents, the degrees of domination were 25% and 50.6%, respectively.

In all studied segregating hybrid crosses, hardseededness was found as a rule among black-coated seeds.

Our data are confirmed by Woodworth (1933) and Shahi and Pandey (1982), who studied another experimental material.

The data testify that the genes responsible for hardseededness are linked with black seed coat genes.

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2) Reaction to low temperature of the F1 crosses and their parental forms.

Cold-resistant varieties 'Comet' and 'Amurskaya 41' were used as parental forms. They had been crossed with sensitive-to-low-temperature variety 'Gieso'. Germination analysis of parental components was carried out on plants taken at random, and germination of 50 seeds was established. Under low temperature, germination had unimodal character with different levels of modal class. Sensitive variety Gieso was characterized by maximum frequency of this character within the germination range from 45 to 55%, while in cold-resistant varieties Comet and Amurskaya 41, the modal frequencies fitted germination range from 92 to 98%.

It was determined that all the varieties had normal distribution of the character since $X^2 < X^2 = 3.84$ (Table 1). All the studied parental forms revealed four common classes of germination out of a total of 18 analyzed. Continuous variation of Gieso included 15 classes, while in Comet and Amurskaya 41, it included seven classes of each variety.

Table 1. Correspondence between actual and theoretical distribution of the character in parental forms $\,$

Average	Fre	quency	0-Е	(O-E) ²	(O-E) ²
value	Actual, 0	Theoret., E	0-1	(0-E)	E
		Come	t		
69.5-84.5	16.65	13.94	2.71	7.344	0.527
89.5	9.74	14.78	-5.04	25.402	1.719
94.5	14.11	12.90	1.21	1.464	0.113
99.5	9.50	8.38	1.12	1.254	0.150
	50	50	0	_	$x^2 = 2.509$
		Amurska	ya 41		
69.5-84.5	16.59	15.18	1.41	1.988	0.131
89.5	12.21	17.26	-5.05	25.503	1.478
94.5	15.40	12.56	2.84	8.066	0.642
99.5	5.80	5.00	0.80	0.640	0.128
	50	50	0	_	$x^2 = 2.379$
		Gies	0		
14.5-34.5	9.01	8.32	0.69	0.476	0.057
39.5	5.56	5.48	0.08	0.006	0.001
44.5	5.92	6.83	-0.91	0.828	0.121
49.5	7.32	7.43	-0.11	0.012	0.002
54.5	6.08	7.02	-0.94	0.884	0.126
59.5	5.68	5.78	0.10	0.010	0.002
64.5-84.5	10.43	9.13	1.30	1.69	0.185
	50	50	0	_	$x^2 = 0.49$

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Fisher F criterion demonstrated that variability of the character between Comet and Amurskaya 41 was nonsignificant (F = 1.32 < F $_{0.1}$ = 2.0). Significant differences in seed germination under low temperature had been established between Comet and Gieso, and Amurskaya 41 and Gieso.

The seeds of F_1 plants from crossing of cold-resistant varieties Comet and Amurskaya 41 with sensitive variety Gieso had been divided into two parts. The first part was used for determining germination, the second for obtaining F_2 plants.

Table 2 demonstrates seed germination of the different F_1 plants in three cross combinations. Because of the low number of hybrid plants, non-parametric Vilkonson-Mann-Witney criterion was used since it was not limited by the mode of distribution (Glotov et al., 1982). The analysis of the F_1 reciprocal crosses Gieso x Amurskaya 41 demonstrated that the cold resistance was determined exclusively by nuclear genes. Taking that into account, the seeds of this cross combination were bulked and used as one sample.

Table	2.	Germination	οf	the	F.	plants	under	1 ow	temperature

Plant N	Gieso x Comet	Gieso x Amurskaya 41	Amurskaya 41 x Gieso
1	87.5	93.0	84.4
2	87.5	93.1	86.1
3	88.8	93.8	87.7
4	91.3	96.0	88.0
5	92.3	97.1	89.2
6	92.5	97.5	92.4
7	93.3	-	92.5
8	95.0	-	93.3
9	97.5	-	94.2
10	-	-	94.7

According to distribution of the character, the F_1 formed unimodal curves with maxima above 90%; i.e., they were very close to cold-resistant parental varieties.

Statistical analysis showed that the F_1 crosses were significantly different from Gieso variety. The differences between the F_1 and cold-resistant varieties were statistically nonsignificant (Table 3). Germination percentage of crosses and parental forms demonstrated complete dominance of alleles that increased cold resistance.

Thus, studying the F_1 and their parental forms revealed that cold resistance was controlled by nuclear genes with complete dominance of alleles that increase cold resistance.

Table 3. Distribution of the character in the F1 and parental forms

Variety (cross)	Number of samples	Average value of the character (%)	Dispersion	Standard deviation
Comet (P ₁)	50	90.80 ± 0.94	43.76	6.61
Gieso (P ₃)	50	49.96 ± 1.90	180.16	13.42
$F_1 (P_3 \times P_1)$	9	91.63 ± 1.13	11.46	3.39
Amurskaya 41 (P ₂)	50	89.88 ± 0.81	33.21	5.76
$F_1 (P_3 \times P_2)$	16	92.06 ± 0.97	15.12	3.89

Reference

Glotov, N. B., L. A. Zhyvotovsky, N. V. Hovanov and N. N. Hromov-Borisov. 1982. Biometry. Leningrad University, USSR.

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3) Segregation of the F_2 populations and F_3 families.

To determine the number of genes determining cold resistance, the method proposed by Merezhko (1983) was used. According to the distribution of the F_2 plants, it was concluded that the part of the progenies like a recessive parent averaged 25%. From this, we concluded that parental forms in the crosses differ from each other by the alleles of one gene. Germination at low temperatures and variability values of the character are presented in Tables 1 and 2.

The actual and theoretical value of germination of hybrid populations appeared to be very close. Thus, the suggested hypothesis regarding differences among parental forms due to one major gene with complete dominance proved correct. The analysis of distribution of the F_2 plants with χ^2 criterion revealed that it differed from normal in both combinations. The normal distribution of the character in parental forms was mentioned above. These results suggest that the mode of distribution in the F_2 is dependent upon genetic factors. A distribution curve of the F2 combination 'Gieso' x 'Comet' has two maxima (Figure 1). The first section of the curve with maximum in the germination range from 50 to 60% was similar to that of cold-sensitive variety Gieso; the second section with range from 96 to 98% resembles a distribution curve of cold-resistant variety Comet. Hence, it follows that hybrid progenies in the F_2 segregated into two phenotypic classes according to their sensitivities to low temperature. Some progenies resemble Gieso by their reaction to low temperature; the others resemble Comet variety. It should be noted that segregation of these classes fits 3.5:1. Segregation

Table 1. Segregation of cold resistance in the F2 crosses

Parental forms and F ₂ crosses	Number of studied plants	Average germination (%)	Dispersion	Standard deviation
Comet (P ₁)	50	90.80 ± 0.94	43.76	6.61
Amurskaya 41 (P ₂)	50	89.88 ± 0.81	33.21	5.76
Gieso (P ₃)	50	49.96 ± 1.90	180.16	13.42
$F_2 (P_1 \times P_2)$	87	80.96 ± 2.02	355.87	18.87
$F_2 (P_3 \times P_1)$	336	77.58 ± 1.02	346.06	18.60
$F_2 (P_2 \times P_3)$	62	83.06 ± 2.19	296.42	17.22
F ₂ (P ₃ x P ₂)	262	78.65 ± 1.20	378.60	19.46

Table 2. Comparison of actual and theoretical distribution of cold resistance in the $\ensuremath{\text{F}}_2$

Cross	χ^2 act.	χ^2 theor.
Gieso x Comet	104.32	23.21
Comet x Gieso	43.67	15.09
Gieso x Amurskaya 41	114.97	23.21
Amurskaya 41 x Gieso	21.18	13.28

in the hybrid population from crossing Gieso x 'Amurskaya 41' was the same (Figure 2). Segregation ratio of tolerant and susceptible progenies fits 4.4:1.

Thus, the experimental data of phenotypic segregation in crosses including varieties differing by cold resistance indicated a dominant gene conditioning cold tolerance.

The analysis of some plants in the F_2 families revealed varietal differences in germination at 7°C (Table 3). It is clear that some of the F_2 families include plants with germination close to cold-resistant variety Comet. They also include some susceptible plants.

Segregation by such characters as cold resistance, flower and pubescence color enables us to conclude that all these characters fall into different linkage groups.

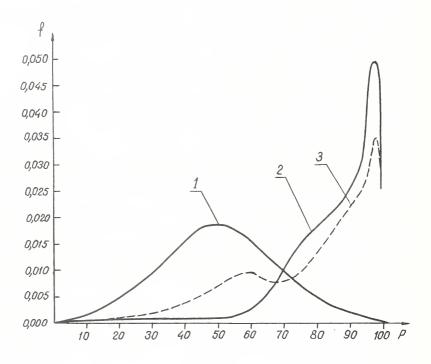


Figure 1. Germinating ability of F_2 crosses and parental soybean varieties at 7 $^\circ$ $$X{\rm -axis}$ - seed germination;

Y-axis - frequency 1 - Gieso; 2 - Comet; 3 - Gieso x Comet

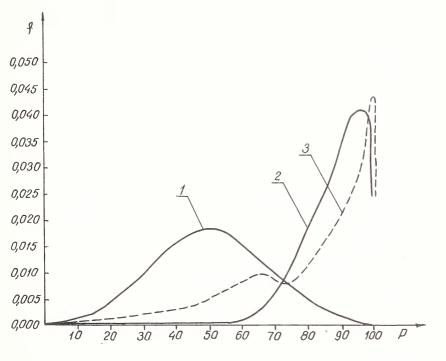


Figure 2. Seed germination of F_2 crosses and parental forms at $7^{\circ}C$ X-axis - seed germination; Y-axis - frequency; $1 - Gieso; \ 2 - Amurskaya \ 41; \ 3 - Gieso \times Amurskaya \ 41$

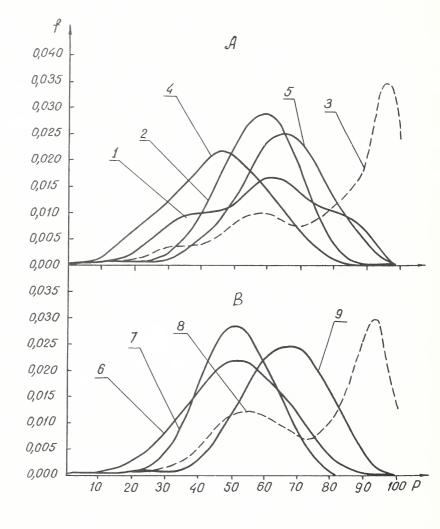


Figure 3. Germination of F_3 families developed from cold-susceptible F_2 plants at 7°

X-axis - seed germination; Y-axis - frequency; A - Gieso x Comet; B - Gieso x Amurskaya 41; 1-9 - No. of F_3 families

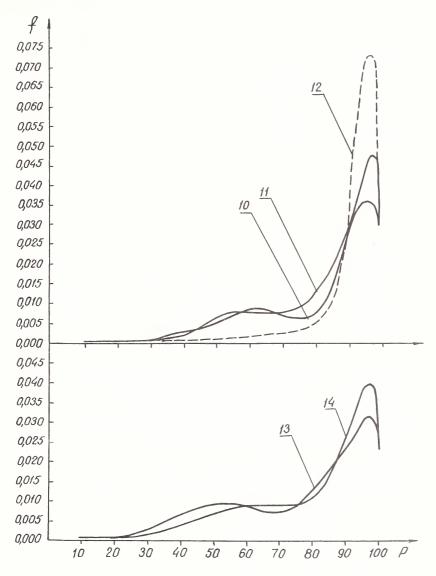


Figure 4. Germination of F_3 families developed from cold-resistant F_2 plants at 7° X-axis - seed germination;

Y-axis - frequency 10-14 - No. of F_3 families

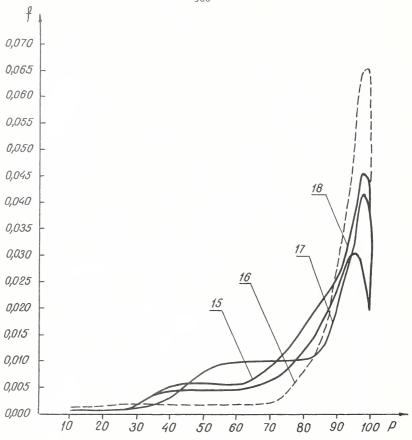


Figure 5. Germination of F_3 families developed from cold-resistant F_2 plant of Gieso x Amurskaya 41 cross at $7\,^\circ$

X-axis - seed germination;

Y-axis - frequency;

15-18 - No. of F_3 families

Table 3. Seed germination of individual F2 plants from cross Comet x Gieso

No. of		- 7°	23°					
plants in the family	No. of seeds	Germination (%)	No. of seeds for germination	Germination (%)				
1	1000	96.0	50	100				
2	50	96	30	96.7				
3	50	94	-	-				
4	100	92	30	100				
5	100	90	30	96.7				
6	100	89	30	96.7				
7	100	87	50	100				
8	100	85	50	96				
9	50	74	25	96				
10	50	58	25	100				
11	80	55	30	100				
12	90	54.4	50	96				
13	100	46	50	100				
14	100	32	50	98				

It was determined that the majority of F_2 plants sensitive to temperature stress were the same in the F_3 (Figure 3). The maximum frequency of the F_3 progenies from families N 4, 6, 7 fits germination range from 44 to 52%, resembling parental Gieso variety. The maxima of the curves 1, 2, 5 and 9 showed germination percentage 60-68%, and were situated on the right side from sensitive variety. In families N 3 and 8, the obvious bimodality of distribution was observed. Thus, it can be supposed that the modal class position changes of the analyzed curves are conditioned by genetic and environmental effects. We supposed the majority of the F_3 families, which were similar to the recessive parent, carry the recessive gene conditioning cold tolerance in homozygous state. Availability of some segregating families is conditioned by the effect of modification factors in the F_2 and F_3 .

The F_3 families N 10, 11, 13, 14, 15, 17 and 18 developed from cold-resistant F_2 plants demonstrated bimodal distribution of the character (Figures 4 and 5). They were heterozygous and showed oligogenic segregation. Families 12 and 16 formed unimodal curves that resembled distribution curves of the dominant parents Comet and Amurskaya 41. Character of distribution is evidence that they carry homozygous dominant gene for cold resistance.

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